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THE ABSORPTION OF ADRENALIN.

By D. MURRAY LYON.

(*From the Department of Therapeutics of the University of Edinburgh, Edinburgh, Scotland.*)

(Received for publication, February 26, 1923.)

Adrenalin, the most powerful of the sympathomimetic amines, acts locally on the terminal mechanism of the sympathetic nerves in the organs and also produces the same response as the sympathetic itself, whether that be stimulation or inhibition (1). It has been shown (2) that the reaction to adrenalin is proportional to the amount of the drug present in the blood at the time, and that the response only continues as long as an excess remains in the blood. Adrenalin disappears rapidly from the circulation and from the tissues, apparently undergoing immediate destruction, and none of it can be discovered in the blood after the reaction is finished (3).

The mode of administration has an important bearing on the character of the response to adrenalin, for its action depends on the rate at which it reaches the tissues, and this varies with the path of absorption. Given intravenously in animals adrenalin causes a rapid rise and fall of systolic pressure, the whole disturbance lasting only a few minutes. With intramuscular injection, the response is still considerable but is slower and lasts somewhat longer. The impression seems to be widely prevalent that adrenalin, given subcutaneously, causes little general effect, and its action is quite uncertain (4–6). This view is probably based on the blanching of the skin which is seen around the site of injection, and on the fact that adrenalin causes vasoconstriction of some vessels. But the spectacular relief from distressing symptoms that occurs in asthmatics within a few minutes after a hypodermic injection of a minute dose of adrenalin has been given, is in itself evidence that absorption by this route is rapid and satisfactory.

When adrenalin is administered hypodermically there develops in the neighbourhood of the needle puncture a white zone which increases in size and persists for a long time. In many cases irregular white lines can be traced for some distance centrally from this area. These lines do not correspond to blood vessels but resemble in distribution the red streaks of inflamed lymphatics which appear in cases of cellulitis. It is suggested that absorption of adrenalin can take place freely by lymphatic channels.

A large number of reactions in the body, *e.g.* the action of enzymes (7), obey the law of mass action, according to which the rate of change is proportional to the concentration of the reacting substance. If a certain quantity of a substance x is being converted into another substance y, the process is rapid at first but becomes slower and slower as the amount of x diminishes.

When the change takes place in two stages (consecutive reactions) the conditions are not so simple. Let x be converted into y, which in turn becomes z. The transference of x into y takes place as above and the conversion of y into z also follows Wilhelmy's law. In this case, however, the rate of formation of z is proportional to the concentration of y in the system at different times, and the quantity of y present at any moment depends on the relative rates of the processes $x \rightarrow y$ and $y \rightarrow z$. The concentration of y starts from zero and increases until a point is reached at which its rate of formation is balanced by the rate of its conversion, after which it again diminishes in amount (8).

The absorption and utilisation of adrenalin are an example of consecutive reactions, the first stage ($x \rightarrow y$) is the introduction of the drug into the circulation, the second ($y \rightarrow z$), its removal from the blood stream into the reacting tissues. The quantity of adrenalin absorbed in unit time is greatest at the beginning of the process and becomes less as the local concentration of the drug diminishes (see Curve X in Text-fig. 2). From the site of inoculation the adrenalin is transferred by lymphatic channels to be discharged at some point into the blood stream. A latent period will be required for its passage between these two points, but this will not affect the character of the reaction, and the rate of the appearance of adrenalin in the circulation will be the same as the rate of its removal from the subcutaneous area.

The quantity of adrenalin available for the tissues at any moment depends on the varying concentration of the drug in the blood stream.

Methods Employed in Interpreting the Records.

If it be assumed that the rate of absorption of adrenalin and the rate of its destruction obey the law of mass action, and that the changes in blood pressure following administration of the drug are

proportionate to the changes in concentration of the substance in the circulating blood, an analysis of the blood pressure curves should yield a good deal of information regarding the processes of absorption and utilisation of the adrenalin.

Let x_0 = the original amount of adrenalin injected.
x_1 = the amount of adrenalin remaining unabsorbed at any time after the reaction has started.
z = the amount of adrenalin destroyed by the tissues.
y = the quantity of adrenalin in the circulating blood.
t = the time which has elapsed since the beginning of the reaction.
r and s = constants which express the rapidity of the two reactions.
e = the mathematical constant 2.7182.

From the law of mass action the rate of transference of adrenalin from the site of injection into the blood stream is given by the differential equation

(1) $$\frac{dx}{dt} = rx_o$$

The rate of utilisation of the adrenalin by the tissues is expressed by

(2) $$\frac{dz}{dt} = sy$$

and the difference between these two processes, absorption and destruction, shows the rate of accumulation of adrenalin in the circulating blood.

(3) $$\frac{dy}{dt} = \frac{dx}{dt} - \frac{dz}{dt} = rx_o - sy$$

By integration from these formulas can be found the actual distribution of the adrenalin between subcutaneous tissue, blood, and reacting tissues at any instant of time.

(4) $x_1 = x_o e^{-rt}$ gives adrenalin still unabsorbed,

(5) $$z = \frac{x_o r s}{s - r}\left(\frac{1 - e^{-rt}}{r} - \frac{1 - e^{-st}}{s}\right)$$ the amount already destroyed,

(6) $$y = \frac{x_o r}{s - r}\left(e^{-rt} - e^{-st}\right)$$ the quantity of adrenalin in the circulating blood.

Since the adrenalin is quantitatively used up in producing its effects, the blood pressure curve should follow the changes in the rate of destruction of the substance and should correspond to formula (2) given above.

(7) $$\frac{dz}{dt} = sy, \text{ or giving } y \text{ its value,}$$

$$= s\frac{x_o r}{s-r}\left(e^{-rt} - e^{-st}\right)$$

that is, the rate of transference of adrenalin to the tissues $\left(\frac{dz}{dt}\right)$ is proportional to the quantity of the drug in the circulating blood, and is simply this value (y) multiplied by the reaction constant (s).

In equation (7) the figures for x_0, t, and e are already known and it only remains to discover values for the constants r and s. This can be done by an examination of the curve itself. Take readings of the curve at four points separated by equal intervals of time. Let the value of these points be represented by A, B, C, and D, and the time interval be p. (The calculation can be considerably shortened by taking A as the initial point in the reaction and giving it zero value.)

The constants r and s are found thus:

$$F = e^{-(r+s)p} = \frac{BD - C^2}{AC - B^2} \qquad \text{If } A = O,$$

$$G = e^{-rp} + e^{-sp} = \frac{AD - BC}{AC - B^2} \qquad F = \frac{C^2 - BD}{B^2}$$

$$H = e^{-rp} - e^{-sp} = \sqrt{G^2 - 4F} \qquad G = \frac{C}{B}$$

$$2e^{-rp} = G + H \qquad 2e^{-sp} = G - H$$

$$e^{-rp} = \tfrac{1}{2}(G + H) \qquad e^{-sp} = \tfrac{1}{2}(G - H)$$

$$rp = w \qquad sp = v$$

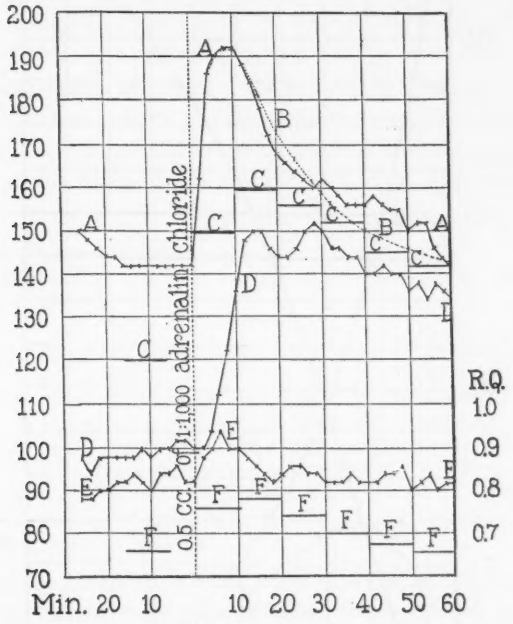
$$r = \frac{w}{p} \qquad s = \frac{v}{p}$$

w and v can be obtained from tables of exponentials. With these newly found constants the blood pressure curve can be reconstructed and compared with the observed figures; the value of the expression $s\frac{x_0 r}{s-r}$ being obtained from $\frac{B - A\,\frac{1}{2}(G-H)}{H}$ or when $A = O$, from $\frac{B}{H}$.

If the calculated and the observed figures correspond closely the values of s and r are satisfactory and they may be employed in formulas (1) to (7).

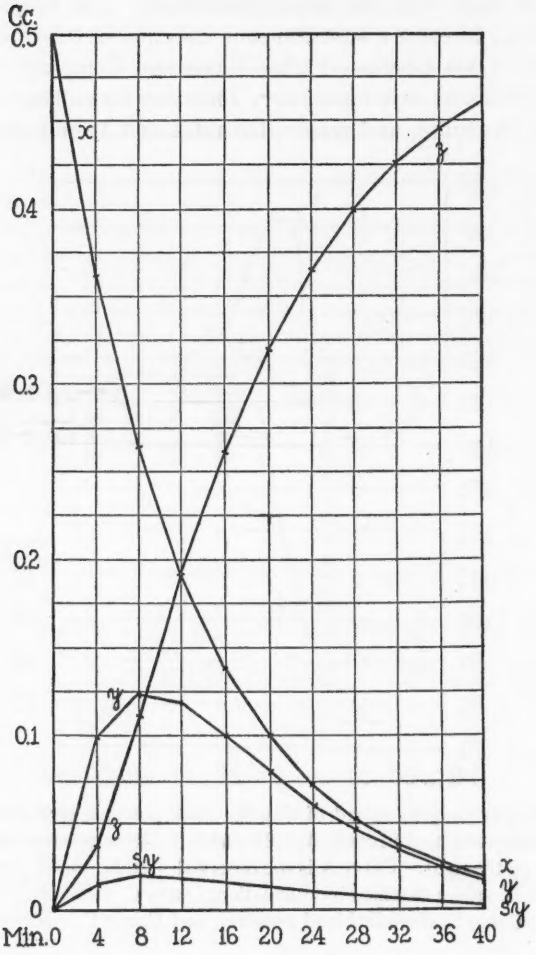
The response to hypodermic injection of adrenalin has been investigated in about 50 patients. Records of the pulse rate, systolic and diastolic blood pressure readings, and the basal metabolic rate

were first made with the usual precautions. The subject was then given in the forearm a subcutaneous injection of 0.5 cc. of adrenalin chloride 1:1,000 solution (Parke, Davis and Company). Following this, observations were made every 2 minutes for an hour on the blood pressure, the pulse, and respiration rates and 10 minute samples of



TEXT-FIG. 1. Graphic record of Case C. The abscissæ give time in minutes, the ordinates refer to Curves A, B, C, D, and E. The respiratory quotient values are shown on the right. Curve A is the observed systolic blood pressure in mm. Hg; Curve B, values obtained by calculation; Curve C, metabolic rates; Curve D, pulse rate; Curve E, diastolic blood pressure; and Curve F, values of respiratory quotient.

expired air were collected over the same period. The effects of the drug included a rise and subsequent fall in the volume of expired air, the oxygen consumption, the carbonic acid output, the metabolic rate, the respiratory quotient, and the systolic blood pressure. The movements of these readings coincide closely and the record of the



TEXT-FIG. 2. Graphs from Case C in which $r = 0.08$ and $s = 0.16$. These lines show the distribution of adrenalin during 40 minutes following its administration subcutaneously. x = adrenalin subcutaneously; y = adrenalin in the blood stream; z = adrenalin destroyed in reacting tissues; and sy = adrenalin passing from blood to tissues.

systolic pressure changes may be taken as expressing the general reaction.

Text-fig. 1 is shown as an example of the blood pressure readings which follow the administration of adrenalin. Curve *A* is fairly smooth for the first 28 minutes but after this it becomes irregular. The constants extracted by the four point method from the regular part of the curve have the values 0.08 for r and 0.16 for s. From these figures the curves of x, y, z, and sy have been calculated (Table I) and these are shown graphically in Text-fig. 2. It will be observed that x declines at first much more rapidly than z rises so that the amount of adrenalin in the circulation increases for 8 minutes. At this point the rates of the absorption and of the removal of ad-

TABLE I.

Case C. $r = 0.08$; $s = 0.16$.

t.....	0	4	8	12	16	20	24	28	32	36	40
x	0.5	0.363	0.264	0.191	0.139	0.101	0.073	0.053	0.038	0.028	0.018
y	0	0.099	0.124	0.118	0.100	0.081	0.063	0.048	0.036	0.026	0.017
z	0	0.038	0.112	0.191	0.261	0.318	0.364	0.399	0.426	0.446	0.465
$x_0 - x_1$	0	0.137	0.236	0.309	0.361	0.399	0.427	0.447	0.462	0.472	0.482
sy	0	0.016	0.020	0.019	0.016	0.013	0.010	0.008	0.006	0.004	0.003

t = time in minutes; x, adrenalin remaining unabsorbed; y, adrenalin in the circulation; z, adrenalin destroyed in the tissues; $x_0 - x_1$, amount absorbed; and sy, quantity in action at the moment.

renalin from the blood equal each other and the drug ceases to accumulate in the blood. At the maximum point, 25 per cent of the original dose of adrenalin is being carried in the circulating blood, but the amount transferred to the tissues at the same time, and available for producing an effect there, is only 4 per cent. The removal of the adrenalin from the site of inoculation is very rapid; after 9 minutes only 50 per cent remains locally, at 20 minutes 20 per cent, and by 40 minutes less than 4 per cent is left unabsorbed. Individual cases show wide differences in their behavior to adrenalin and the records vary considerably in character, notably in regard to the rate of onset, the magnitude of the reaction, and its duration.

In the majority of cases the rise of the blood pressure commenced within 2 minutes after the hypodermic injection, but occasionally the

increase was not evident for several minutes; in one instance a latent period of 10 minutes elapsed (*vide infra*). The increase of pressure may occur quickly or slowly so that the highest point is often reached in 6 to 10 minutes or may be delayed for nearly 40 minutes. If the process of absorption is rapid a greater quantity of the drug can come into action at one time and consequently the apex of the curve is high. This high level does not persist for long and a speedy fall of pressure usually follows. A sustained maximum and a slow decline characterise those cases in whom the onset is more gradual.

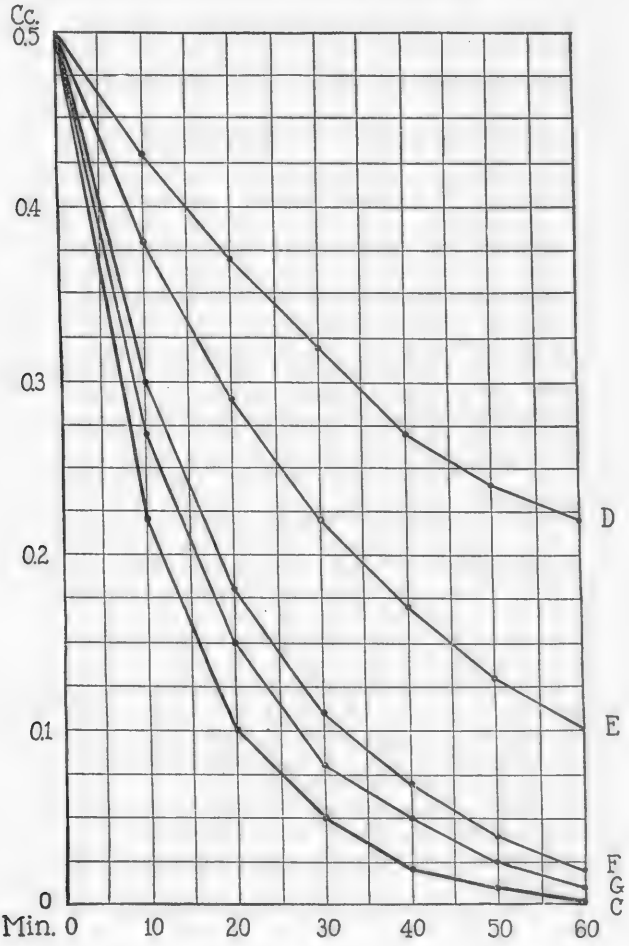
TABLE II.

Adrenalin absorbed $= x_0 - x_1$, or $y + z$.

Case.	Time.							r	s	Time of maximum elevation.	Diagnosis.	Basal metabolic rate.
	0 min.	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.					
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*			*min.*		*per cent*
D	—	0.07	0.13	0.18	0.23	0.26	0.28	0.015	0.15	25	Myxedema.	−36
E	—	0.12	0.21	0.28	0.33	0.37	0.40	0.027	0.12	16	Diabetes.	−15
F	—	0.20	0.32	0.39	0.43	0.46	0.48	0.050	0.30	14	Asthma.	+ 7
G	—	0.23	0.35	0.42	0.45	0.48	0.49	0.060	0.10	13	Cancer.	+ 8
C	—	0.28	0.40	0.45	0.48	0.49	0.50	0.080	0.16	8	Hyperthyroidism.	+20

Two patients suffering from diabetes mellitus offered a great contrast. Both were young men; the one, A, was an excitable individual who was at the beginning of his course of treatment, the other, B, was of plethoric temperament and had been sugar-free for a year. In A the reaction took place with startling rapidity, the pressure mounting at once and the maximum increase (52 mm. Hg) occurring at 10 minutes. The decline was almost equally speedy and within 24 minutes the reaction was practically over. The other subject, B, showed a very different type of response. Following the injection of adrenalin there was a latent period of 10 minutes during which the blood pressure remained steady. A quite insignificant rise then took place (10 mm. Hg) and persisted for some time. A clue to the cause of the difference between the responses of A and B was obtained from the pulse rate. B had a habitual bradycardia (48 beats per minute) while A's heart usually ran about 80. An investigation by Professor Meakins of the blood flow in these two subjects gave similar information. While B's circulation rate was less than normal, A had an accelerated blood flow. It would seem from these cases that the rate of absorption of the substance from the subcutaneous tissues into the blood was dependent on the rate of the blood flow. The effect of rapid circulation rate on the rate of absorption would also serve to explain the exaggerated reactions to

adrenalin which are obtained in the subjects of exophthalmic goitre, without raising the question of any hypersensitiveness of the tissues to adrenalin in such cases. A similar view has been put forward by Kendall (9).



TEXT-FIG. 3. Rate of absorption of adrenalin in five cases (Table II). The curves, which show the rate of removal of adrenalin from the site of injection, have been calculated from $x_1 = x_0 e^{-rt}$

The values of the constant r are as follows: Case D = 0.015; Case E = 0.027; Case F = 0.05; Case G = 0.06; and Case C = 0.08.

In Table II are set out figures showing the rate of absorption of adrenalin in five cases. The absorption constants (r) range from 0.015 to 0.08 but, with the exception of Case F, the constants (s) controlling the second reaction lie closer together. It will be seen too that the time at which the maximum elevation is reached also depends on the r constant. When this is small the rise is slow, and conversely. Case D was an untreated case of myxedema whose bodily activities were sluggish. This may account for the slow absorption and the comparatively small response to the drug. The results in Table II are graphically shown in Text-fig. 3.

In the above discussion it has been assumed that adrenalin acts in a strictly quantitative fashion in producing its effects. This view, however, must be modified in the light of recent investigations (10). It is known that when the dose of adrenalin is increased by a certain amount, the resultant response is not increased in direct proportion but falls short of expectation. The relationship between the size of the dose and the magnitude of the effect is a logarithmic one corresponding to the formula

$$E = n \log_e s(+c)$$

E = effect.
s = dose of adrenalin.
n = a constant which expresses the activity of the subject.
c = an added constant.

This would modify the above conclusions to a certain extent. Since large doses produce apparently a proportionately less response than the smaller doses, the amount of adrenalin in action at the maximum of the curve must be even greater, and the rate of absorption of the drug will be faster, than the simple case allows for.

In order to make this logarithmic correction it would be necessary to know the value of the constant of reactivity n. To obtain this, the responses to at least two different amounts of adrenalin must be studied. The figure for n can then be extracted from the following formula.

$$n = \frac{E_2 - E_1}{\log_e s_2 - \log_e s_1}$$

when s_1 and s_2 represent the two doses employed and E_1 and E_2 are the maximum values of the responses to these doses.

It seems highly probable that the value of the reactivity constant n would give a better idea of the sensitiveness of the individual to adrenalin than the method at present in general use (Goetsch's).

SUMMARY.

1. Adrenalin solution given subcutaneously is usually rapidly absorbed, probably by lymphatic channels.

2. The speed of this process may be influenced by the circulation rate.

3. The relative amounts of adrenalin at any moment unabsorbed at the site of inoculation, carried in the circulating fluids, and taken up by the reacting tissues can be calculated from figures extracted from the curve of the blood pressure changes. The relative rates of transference of adrenalin into the blood and from the circulation into the tissues can also be estimated.

4. When absorption takes place rapidly a large quantity of the drug comes into action at once and the maximum occurs early, the curve of blood pressure reaches a considerable height, and subsides quickly. When absorption is slow the apex appears later and does not reach so high a level.

5. The response to adrenalin bears a logarithmic relationship to the dose employed and a method of allowing for this is indicated.

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CONTRIBUTIONS TO THE BIOLOGY OF TISSUE CELLS.

I. The Relation of Cell Crowding to Tissue Growth in Vitro.

By ALBERT FISCHER, M.D.

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Plate 37.

(Received for publication, April 30, 1923.)

INTRODUCTION.

When small bits of tissue are cultivated *in vitro*, do they persist and the cells multiply indefinitely? As a first step toward answering this question the attempt was made to determine whether it was possible to obtain growth and multiplication from one single cell with the ultimate development of cell colonies comparable to those formed in the cultivation of bacteria.

It was necessary to develop a method for isolating living cells from tissues *in vivo* or *in vitro*. Under both conditions the cells are strongly adherent to one another by protoplasmatic connections. These anastomoses between the cells in the organism vary with the different tissues and are very strong in tissues which have a vital significance. In culture, the cells are, in addition, connected by means of fibrin threads in the clot. It is impossible to separate out an individual cell from an ordinary culture with a knife or other instrument; the injury is too great. To overcome the difficulties, a method for isolating cells from tissue cultures was worked out by Rous.[1] He digested the tissue culture by means of trypsin and finally obtained a suspension of tissue cells. A different technique has been used in the present study.

Method 1.

Ordinary absorbent cotton is finely cut between two fingers, by means of a small pair of scissors, to a fine "powder" and sterilized

[1] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 549.

by dry heat. Cultures are then made in the following way. A small drop of fresh concentrated embryonic tissue juice is placed on a sterile cover-glass; a small amount of the finely cut cotton is stirred into the drop with the point of a needle or cataract knife in order to spread out the drop. The small short cotton threads will then form a rather compact meshwork in the drop. A small piece of tissue is cut out from another culture, placed in the meshwork of cotton, and the drop stirred a little. The cotton threads seem to have the ability to gather around the piece of tissue and a good contact to the supporting apparatus is established. The cover-glass is placed on the slide, sealed with paraffin, and brought into the incubator. After 24 hours in the incubator a large mass of cells may be observed growing on the cotton threads. At the start, a *single layer* of cells covers the cotton threads; later on they fill up the spaces between the threads (Fig. 1).

Connective tissue cells show a typical spindle shape, particularly those in the closest contact with the cotton threads, but toward the middle of the fluid medium spherical forms may be observed. When the time comes to transfer these cells as isolated individuals to a new medium, the cover-glass is *unsealed* and the *excess* fluid which contains the decomposing products formed during the growth is disposed of by aspiration with a fine pipette, and a little Locke's fluid containing 1/8 per cent gelatin is carefully added to preserve the fragile cells from injury.[1] The cells having been washed once this way, a new drop of Locke's fluid is added and the drop containing the cotton threads, with the adherent cells, is carefully aspirated and expelled on a cover-glass on which is a drop of fresh plasma. The cells are stirred with a needle and a drop of fresh embryonic tissue juice is added and stirred in. After a short interval the plasma coagulates upon the preparation, fixing the cells in place.

RESULTS.

When examined under the microscope the cells will be seen to have assumed a spherical form, and if the cotton culture from which they were transferred is also examined, it is apparent that all the cells which eluded the pipette have become spherical. This same phenomenon

has also been already described by Rous[1] and Uhlenhuth.[2] The picture now presented looks very much like a mass of leucocytes.

Upon closer observation of the individual fibroblasts it is possible to perceive an active ameboid movement. Pseudopodia of rounded outline are put out and retracted, and active currents pass through the granulated protoplasm. Broad tongue-like pseudopodia can be seen without any granules into which, after a time, the granules suddenly flow. These granules may have a remarkable rate of speed.

As a rule, after a few hours, the cells will have assumed a typical spindle shape characteristic of fibroblasts. When this change has taken place all movement appears to cease. In this condition the cells remain apparently unchanged in outline but the protoplasm fills up gradually with vacuoles and fat granules, death takes place, and the protoplasm decays and dissipates.

It has not been possible to observe the division and proliferation of an isolated cell, though hundreds of cells have been studied with this object in view. Growth by proliferation has been observed only when a number of cells were in close contact in a culture.

These observations lead to the question of whether intercellular contact is essential to the cells for their growth and multiplication, and what significance the contact has in the growth and multiplication of the cells.

Before discussing the problem, it will be well to describe other methods by which the attempt was made to determine whether isolated cells are able to multiply. For the results of the experiments just mentioned may have been due to a technical error. Anyway, it has to be taken into consideration that not impossibly the manipulations may injure the cells, despite the signs which seem to indicate perfect vitality.

When ordinary cultures were cut in two halves and transferred to a fresh medium, it was frequently noted that the cells began to grow out first from the center of the cut edge of the culture, that is to say from the point at which the cells were most crowded, and only a little later on from the periphery. This has also been noted by Carrel.[3] From the observation it might be deduced that the central part of the

[2] Uhlenhuth, E., *J. Exp. Med.*, 1915, xxii, 76.

[3] Carrel, A., personal communication.

culture has a greater ability to proliferate than the peripheral part, where the cells are more scattered. Some experiments were undertaken to see how minute the fragments of a piece of tissue from a culture could be and retain the ability to multiply when transferred to a favorable culture medium. It was found that if the transplanted piece consisted of a few scattered cells no growth took place, and the cells took on the aspects of degeneration at a time when the control culture seemed to be in perfect condition. By contrast, when the small clumps of cells had been taken from the central portion of the culture where it was dense, growth was extensive. The tissue used in these experiments was derived from a 4 month old strain of fibroblasts. The small fragments were obtained by clean cuts with a sharp cataract knife. After a little practise fragments could be cut so small that they just could be distinguished with the naked eye.

Here it could be objected that the poor growth of the fragment containing only scattered cells was a result of retraction of the fibrin clot. In the attempt to maintain uniform conditions during the separation of the small fragment from the mother culture, another technique was devised.

Method 2.

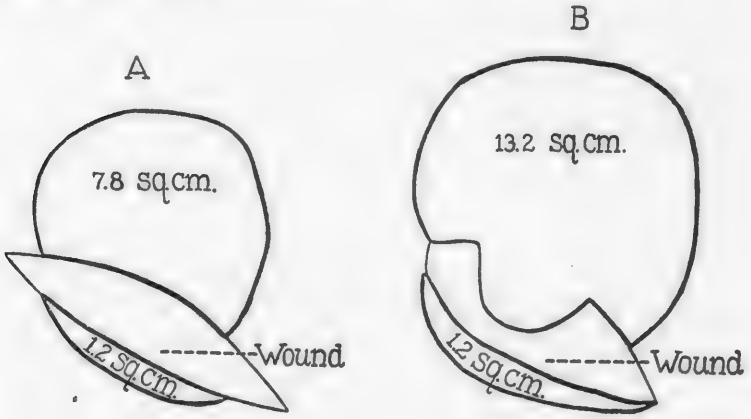
A culture of fibroblasts (the 10 year old strain of The Rockefeller Institute[4]) was permitted to grow for about 20 hours, and then the cover-glass was unsealed and an incision was made in the periphery of the new growth with an ordinary sharp cataract knife, in order to separate from the mother culture a piece of tissue at the periphery where the cells were rather scattered. The cover-glass was replaced on the slide, it was sealed with paraffin, and an outline drawing was made of the tissue—the big piece as well as the small one. The culture was brought back to the incubator for another 30 hours; at the end of the second incubation a new drawing was made and the area measured. It was observed that the mother culture had greatly increased in size, but the fragment, separated from it by means of the wound in the clot, consisted of scattered cells and had rather decreased in size. Fig. 2 and Text-fig. 1 illustrate this experiment. It can be seen from Fig. 2 that a peninsula had grown on the surface

[4] Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv. 755.

of the cover-glass from the central part of the main tissue, but that the separated small piece had not increased in size, much less encroached on the open space.

DISCUSSION.

We are not dealing with the growth of independent cells in the ordinary tissue culture but with a partial organism—a piece of tissue, obeying the laws of regeneration.



TEXT-FIG. 1. *A*, outline drawing of the culture shown in Fig. 2 after incision. The limits of the mother culture, the separated culture, and the wound are to be seen. *B*, outline drawing of the same culture after 24 hours incubation. The mother culture has almost doubled in size since the wound was made but the separated fragment has ceased to grow.

The embryonic tissue juices contain substances which promote growth *in vitro* for an indefinite length of time.[5] Extracts of tissues from the adult organism also possess growth-promoting substances but not of the same grade as the embryonic tissue juices. Carrel[6] has shown that certain tissues give an extract with a greater activating power than others. Extracts of leucocytes or spleen possess nearly as much activating substance as embryonic tissue juice.[7] The

[5] Carrel, A., *J. Exp. Med.*, 1913, xviii, 287.
[6] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.
[7] Carrel, A., *J. Exp. Med.*, 1922, xxxvi, 385.

mechanism of growth and multiplication of tissue cells is a profound and complex phenomenon. Besides the growth-promoting factors found in the tissue juices, there may possibly be something, produced in the body of the cell or certain cells only, which initiates cell division and is transported directly from one living cell to another.

The cytotropic character of tissue cells has already been noted by several investigators. Roux[8] was the first to describe it. Recently Rous[1] has noticed the striking ability of isolated tissue cells to reunite. He never saw any division or multiplication of isolated single cells. Also Burrows[9] mentions the important relation of cells in tissue cultures to each other. He expresses himself as follows: "The tissue culture cannot be compared in detail with the bacterial culture. The tissue cells planted in plasma do not grow at the expense of the plasma. Single cells may show movement in this medium but they do not grow. . . . . Growth takes place only about fragments of tissue. . . . . The nutrient material for the growth of these cells comes from the cells disintegrating within the fragment."

SUMMARY.

1. A method has been described by which it is possible to scatter isolated tissue cells in a suitable medium.

2. Experiments were undertaken to determine whether minute fragments of fibroblastic culture are able to persist and multiply when transferred into a new culture medium.

3. Cell divisions were not observed in isolated cells under the conditions of the experiment. Growth took place only when the tissue cells were numerous and close to one another.

EXPLANATION OF PLATE 37.

FIG. 1. Fibroblasts growing in a meshwork of cotton threads in a drop of embryonic tissue juice.

FIG. 2. A culture of fibroblasts at the end of 48 hours incubation. After 24 hours incubation the culture chamber had been opened and an incision made in the clot whereby a small part of the edge of the culture was separated from the mother portion. The culture was resealed and incubated again, stained, and photographed. It can be seen that whereas a peninsula of cells has grown into the wound from the mother culture, the separated tissue has failed to increase.

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[8] Roux, W., *Arch. Entwcklngsmechn. Organ.*, 1894, i, 161; 1896, iii, 127.

[9] Burrows, M. T., and Suzuki, Y., *J. Immunol.*, 1919, iv, 1.

(Fischer: Biology of tissue cells. I.)

STUDIES ON VARICELLA.

The Susceptibility of Rabbits to the Virus of Varicella.

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Plates 38 to 40.

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In spite of the ignorance regarding the etiological agents concerned in the contagious diseases of children, it has been possible, through the careful clinical observations of such men as Sydenham, to differentiate one from the other. Notwithstanding this wide clinical knowledge, it is still impossible to control the spread of these diseases, or to arrest their course once they have established themselves in susceptible individuals. It is true that some progress has been made in the prevention of measles, scarlet fever, and chicken-pox by the introduction of the method of injecting serum from convalescent patients into exposed individuals. Attempts have also been made to prevent chicken-pox by vaccination with fresh vesicle lymph, and while a few workers have reported positive results with this method, the majority of investigators have not succeeded. It is evident that these procedures involve objectionable features which would detract from the usefulness of the methods were an attempt made to apply them widely. To develop better methods of prevention, it is highly desirable to transmit the diseases to animals in such a way as to obtain a concentrated virus similar to vaccine virus or the virus of rabies.

Varicella is usually considered a mild disorder, but, nevertheless, for many reasons, it has appeared to be a suitable one on which to begin study.

In the first place, chicken-pox, in spite of its mildness, ranks high among the infectious diseases as a cause of loss of time from schools, and is very troublesome in institutions, such as orphan asylums and hospitals for children. Also, knowl-

edge concerning chicken-pox would probably be directly applicable to the more severe disease, smallpox. Although this disease no longer prevails to the wide extent that it did in former centuries, nevertheless, it is still common in certain sections of the United States. According to Low (1), for the last 20 years there has been an average of 20,000 to 30,000 cases of smallpox every year. Recently, in 1 year, over 94,000 cases (2) were reported. Usually it is not difficult to differentiate smallpox from chicken-pox, but there are instances when it is not easy, and mistakes undoubtedly occur. Although Paul (3), Tièche (4), Force and Beckwith (5), Salmon (6), and Paschen (7) have introduced certain laboratory methods of aid in diagnosis, these are not entirely satisfactory. Furthermore, in chicken-pox there is a characteristic skin lesion which may serve as a mark of identification in case the disease were produced in animals. Although the recent work on herpes and lethargic encephalitis has aroused great interest in all diseases in which vesicular lesions on the skin are present, relatively little experimental work has been done on varicella.

There are other diseases, such as sheep-pox, vaccina, smallpox, alastrim, herpes zoster and symptomatic herpes, with which it is possible to compare chicken-pox. Certain of these diseases have been grouped under such general terms as "*ectodermoses neurotropes*" by certain investigators, for instance, Levaditi and Nicolau (8) and Lipschütz (9). From the numerous studies on these closely related diseases many facts are available which may be of aid in work undertaken with varicella. Upon first thought there seems to be little connection between sheep-pox and lethargic encephalitis in man. On considering all these diseases in a series, however, (Table I), studying the clinical relations existing between them and taking into consideration the results of the experimental studies which have been made, the two just mentioned do not seem so far removed from each other.

It is now generally believed that chicken-pox and smallpox are distinct and different diseases. This has not always been the case, however, and as late as the middle of the 19th century Hebra (10) taught that they were identical. Even at the present time there is a difference of opinion in regard to the relation of smallpox and varioloid to alastrim on the one hand, and of chicken-pox to alastrim on the other. Von Bókay's (11) paper on the relation of chicken-pox to herpes zoster appeared in 1909, and since then a number of other papers have appeared in which the idea that the two diseases are identical has been supported or opposed. For many years there has been much discussion concerning the interrelationship existing between the various kinds of herpes, and this interest has been stimulated recently by the work of Doerr (12) and others on herpes and lethargic encephalitis. If symptomatic herpes and lethargic encephalitis are caused by the same etiological agent, then the viruses of all the diseases listed in Table I, with the exception of chicken-pox and herpes zoster, have been recovered in some form amenable to experimentation.

Some attempts to transmit varicella to animals and to normal children have already been made. Salmon (6), Tyzzer (13), and Teissier, Gastinel, and Reilly (14) were unable to obtain a specific reaction on rabbits' corneas inoculated with

fresh fluid from varicella vesicles. Swellengrebel (15) found cellular changes in rabbits' corneas inoculated with vesicle lymph, but these changes were not considered characteristic of chicken-pox. Bertarelli (16) and Gins (17), however, reported that, in rabbits' corneas inoculated with fresh vesicle lymph, cellular changes occurred which were specific for varicella and were not present in the controls. Levaditi (18) found Guanieri bodies in rabbits' corneas inoculated either with vaccine virus or with varicella lymph. Park (19), Martin (20), and Tyzzer (13) were unable to transmit chicken-pox to monkeys. Hess and Unger (21) failed to produce varicella in normal children by inoculating them upon the mucous membranes of the nose and throat with vesicle lymph and material collected from the nose and throat of patients with chicken-pox, or by inoculating them intracutaneously, subcutaneously, or intravenously with fresh vesicle lymph.

TABLE I.

An Arrangement of Certain Diseases to Show How Closely They May be Related as Evidenced by Reports of Clinical Observations and of Experimental Work.

	Sheep-pox.
	Horse-pox.
1	Cow-pox.
	Smallpox.
2	Varioloid.
3	Alastrim.
	Chicken-pox.
4	Herpes zoster.
5	Symptomatic herpes.
6	Lethargic encephalitis.

Many workers have attempted to vaccinate normal children against varicella, using fresh vesicle lymph for this purpose. Although most observers[1] have reported only negative results, Steiner (22), Kling (23), Lapidus (24), Meyer (25), Gyr (26), Hotzen (27), and others have reported that the inoculations were followed by positive results in certain cases. These investigators state that after such inoculations some of the children had mild chicken-pox with no local reaction; in other children a local reaction and a mild chicken-pox occurred; in others only a

[1] See Tyzzer's paper for further references.

local reaction at the site of inoculation appeared; and in still others, often the largest group (26, 27), no reaction of any kind was noticed. All of the writers agree that the vesicle fluid should be collected during the first few days of the eruption and used shortly afterwards. Kling (23) stated that to obtain positive results at least six inoculations should be made, and that even then only one may be successful. These reports seem to indicate that the virus in the vesicles either is very dilute or has lost its virulence.

In view of the conflicting reports by various investigators who used vesicle fluid in their experimental work on the transmission of varicella to man and to animals, it seemed advisable to search elsewhere in the body for the virus of chicken-pox in the hope that thence it might be obtained in a condition more suitable for experimental purposes. Certain observations soon afforded indirect evidence that the virus occurs in the blood and that the amount there does not necessarily correspond to the number of skin lesions. It was noticed that irritation seemed to have a direct influence on the localization of the virus in the skin as evidenced by the appearance of an unusual number of vesicles at the site of irritation. The picture was particularly striking when the irritation involved the skin of the face and extremities, parts of the body usually least affected by the eruption of chicken-pox.

The effect of irritation on the localization of the virus in the skin has been observed in 5 of 51 patients. A brief summary of these observations follows.

B. K. had adhesive plaster applied to the ankles to alleviate the pain of chronic arthritis. After the patient developed varicella the adhesive was removed. Beneath it were found more vesicles than on all the rest of the body (Fig. 1).

B. S and J. M. wore napkins which irritated the skin and the eruption localized mostly in the irritated areas (Figs. 2 and 3).

J. G. wore soft collars with his necktie drawn very tight. Just beneath where the tie rested was a band of vesicles extending around the neck (Fig. 4).

V. S., a young adult, had acne over the face and upper part of the back. The eruption was most marked over these areas and actually involved the acne lesions.

From reports in the literature it was found that the eruption of many of the exanthemata can be made to localize by irritation, provided the irritant is applied before or shortly after the appearance of the eruption. Von Pirquet (28) and Schick (29) have demonstrated this phenomenon in measles, Heim and John (30) in scarlet fever, Swoboda (31) in chicken-pox, and Hebra (10), Tièche (32), and many others in smallpox. Hebra also noticed that in patients with ichthyosis the eruption of smallpox occurred only on the scrotum and on other parts of the body where the skin was fairly normal. Calmette and Guérin (33), Gins (34), Camus (35), and Levaditi and Nicolau (8) have shown that vaccine virus injected intravenously into rabbits can be made to localize in the skin if certain areas be injured immediately after the injection by shaving or pulling out the hair. Camus (35) demonstrated that the localization of the virus under these conditions is not dependent on the presence of injured tissue, but upon the dilatation of the small vessels in the skin.

The observations on patients mentioned above indicate that the virus of chicken-pox probably is present in the blood, and it was thought that it might be possible to demonstrate the disease in animals by injecting them with patients' blood, then shaving and irritating the skin. Before proceeding, however, with the experimental work on varicella, it seemed advisable to determine whether there is a practical method of demonstrating vaccine virus in the blood of animals previously inoculated on the skin. The following experiment, therefore, was performed.

Rabbit A was shaved on both sides of the body. The skin was scarified gently and then inoculated with vaccine virus.[2] A confluent eruption occurred at the site of inoculation. 4 days after the inoculation the skin over the thorax was dissected back,[3] and 10 cc. of blood were removed from the heart. This blood was injected immediately into the ear vein of Rabbit B. A small portion of skin was shaved and scarified shortly after the injection. 5 days later several nodules (Fig. 5) appeared in the shaved skin. These developed into typical vaccine pustules. 2 weeks after the lesions had healed the rabbit was vaccinated on the skin and was found to be immune.

Although the above experiment showed that vaccine virus can be recovered from the blood of animals, it has been impossible to demonstrate in the same way the presence of the virus of varicella in the blood of patients. Other methods, then, were sought. In view of the fact that Pasteur (36) was able to grow the virus of rabies in the brain

[2] The vaccine virus used in this work was supplied by Dr. Noguchi.

[3] All operations were performed under ether anesthesia.

of animals, and that a similar method has recently been employed successfully in the growth of herpes virus, an attempt was made to demonstrate the virus of chicken-pox by injecting small quantities of fresh blood and vesicle fluid from patients directly into the brain of young rabbits. Only negative results were obtained with this method. Then, since the testicles of rabbits have already been shown to be a suitable place for the growth of spirochetes (37, 38), vaccine virus (39), and tumors (40), they were thought of as possibly being a suitable place in which to grow and concentrate the virus of varicella.

Further evidence of the advantage of using the testicles as a place to cultivate a virus was found in the work of Ohtawara (41). He recently reported that he was able to demonstrate vaccine virus in the blood of rabbits vaccinated on the skin by injecting the blood into the testicles of normal rabbits. We have confirmed his work.

Rabbits were vaccinated over extensive areas on both sides of the body. 4 days later the skin over the thorax was dissected back, blood was removed from the heart, and 1 cc. was injected into each testicle of several normal rabbits. After 4 days the testicles were removed, ground up with sand, and tested for the presence of the virus by application of the emulsion to the shaved skin of normal rabbits. Confluent eruptions were obtained with the testicular material, whereas 1 cc. of blood, similar to that injected into the testicles, caused no visible reaction when ground up and smeared directly on the scarified skin.

The demonstration of vaccine virus in the blood of rabbits inoculated on the skin was relatively easy because the virus (39) used in the experiment had been cultivated in the testicles of rabbits for a number of years and can now be transferred without difficulty from rabbit to rabbit by testicular inoculation. According to Dr. Noguchi,[4] however, difficulty was encountered in the original cultivation of the virus in the testicles and it was not until after the fifth or sixth successful passage of the virus that he was certain it could be transferred indefinitely from rabbit to rabbit in this manner. In view of Noguchi's experience with vaccine virus one might expect to encounter trouble in demonstrating the presence of the virus of varicella in the blood of patients by injecting the blood into the testicles of rabbits. It was decided, therefore, in working with this unknown virus to use the well

[4] Personal communication.

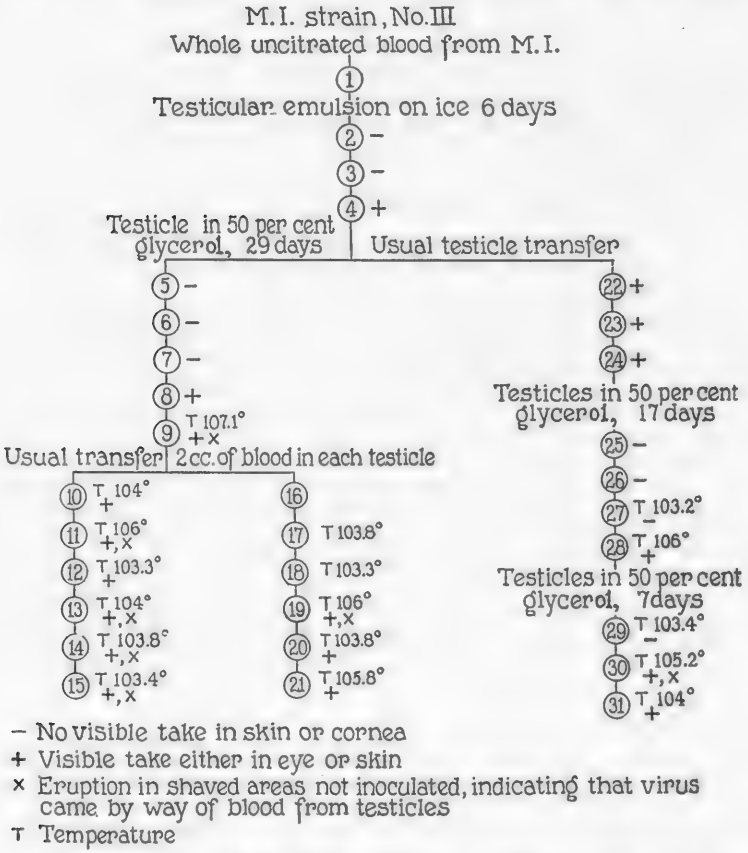
known laboratory procedure of increasing the virulence of a virus by passing it through a number of animals. The time of transfers is important, for if they are made too early a sufficient growth of the virus to prevent its loss eventually by dilution may not have occurred and if they are made too late the virus may be either dead or damaged to such an extent that it becomes weaker with each passage and finally dies. Plans were made, therefore, to inject blood from a number of patients with varicella into the testicles of rabbits and to make repeated transfers from testicle to testicle at various intervals of time, in the hope that in a few instances the virus would become concentrated enough to manifest itself in the shaved skin. Intervals of 4 days were first chosen for the transfers. This choice was made arbitrarily because the proper time to transfer vaccine virus from rabbit to rabbit is at intervals of 3 to 4 days. In working with the virus of varicella, it proved unnecessary to try other intervals, as every 4 days was soon found to be the correct time for the transfer of this virus also.

The details of the method employed in recovering a virus from the blood of varicella patients are as follows: Blood was drawn from patients with chicken-pox usually during the first 24 hours after the appearance of the eruption. The blood was not citrated and before clotting occurred was injected in 2 cc. amounts into each testicle of normal rabbits (1,800 gm.). These large quantities of blood were used intentionally. At the time of inoculation the needle was moved about in the tissues to produce a certain amount of trauma. 4 days later the testicles were removed, ground up thoroughly with sterile, chemically clean sand, and mixed with 10 cc. of physiological salt solution. The mixture was allowed to stand until the sand settled to the bottom. Strict asepsis was observed throughout the work. Portions of the testicular emulsion were tested for the presence of ordinary bacteria by means of cultures on blood agar, in broth, and in Smith-Noguchi tubes. Other portions for future use were stored on ice either in the original state, or after the addition of equal quantities of glycerol. Then 1 cc. of the emulsion was injected into each testicle of normal rabbits. Two areas on the rabbits' skin were shaved and scarified. One of the areas was smeared with the emulsion, the other was used as a control. An eye of each rabbit was also inoculated.

Both corneas were scarified with a cataract knife (cocaine anesthesia was always used); one was inoculated with the testicular emulsion, the other was used as a control. The first few animals in each series showed little reaction other than that which might be expected to follow the trauma of the inoculations. The skin and cornea healed rapidly. The scrotum was edematous at times for 24 to 48 hours. The testicles, when removed, were slightly swollen. Necrotic areas and often remains of the material injected studded the tissue in various places. This reaction was no more striking than that caused by the injection of an emulsion of normal testicles. The first few rabbits in each series and all the rabbits in the series from which no virus was recovered served as excellent controls for the work. After four to eight transfers of the virus from rabbit to rabbit, however, in certain series, the testicles became tense and firm on the 3rd or 4th day after the inoculation, and the scrotum often remained edematous. In the inoculated eye a roughness of the cornea and a circumcorneal redness appeared on the 3rd or 4th day following the inoculation. An erythema and swelling not present in the control occurred along the lines of scarification in the skin 4 to 6 days after the inoculation (Fig. 6). The testicles, when removed from the animals, were usually swollen and hemorrhagic. Later, when the virus became more concentrated, the inoculated animals looked sick, refused to eat, lost weight, and occasionally developed a diarrhea. At first the temperatures of the rabbits were not elevated. Later, however, it was not unusual for the rabbits to have temperatures of 106–107°F. on the 3rd or 4th day after the inoculation. Temperatures of 104° and 105° occurred frequently (Text-figs. 1 to 5). Occasionally, in addition to the reaction in the skin inoculated with the testicular emulsion, discrete, papular lesions appeared 5 to 11 days after the inoculation in the control areas (Fig. 7). This phenomenon seemed to indicate that the virus invaded the blood stream of the rabbits and became localized in the irritated skin. Invasion of the blood stream was then demonstrated by removing blood from these rabbits and injecting it into the testicles of other rabbits (Text-figs. 1, 2, *b*, and 5, *b*).

Blood from eleven patients with varicella was injected into the testicles of rabbits and transplants from each were made through at

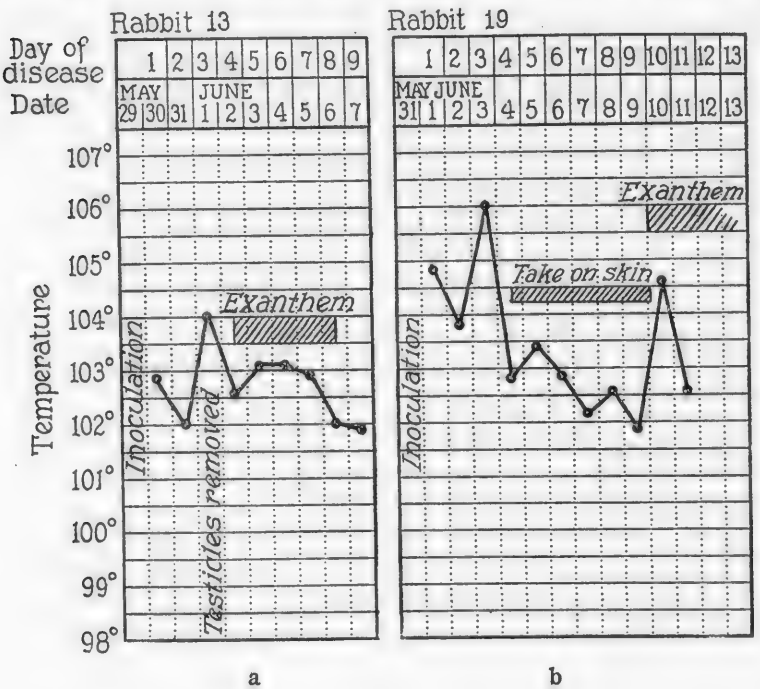
least eight rabbits. It was possible in this way to recover a virus from five of the patients. Only the results obtained with the blood


M.I. strain, No. III
Whole uncitrated blood from M.I.
1
Testicular emulsion on ice 6 days
2 –
3 –
4 +
Testicle in 50 per cent glycerol, 29 days
Usual testicle transfer
5 –
6 –
7 –
8 +
9 T 107.1° +×
Usual transfer 2 cc. of blood in each testicle
10 T +104°
11 T +,× 106°
12 T + 103.3°
13 T +,× 104°
14 T +,× 103.8°
15 T +,× 103.4°
16
17 T 103.8°
18 T 103.3°
19 T +,× 106°
20 T + 103.8°
21 T + 105.8°
22 +
23 +
24 +
Testicles in 50 per cent glycerol, 17 days
25 –
26 –
27 T – 103.2°
28 T + 106°
Testicles in 50 per cent glycerol, 7 days
29 T – 103.4°
30 T +,× 105.2°
31 T + 104°
– No visible take in skin or cornea
+ Visible take either in eye or skin
× Eruption in shaved areas not inoculated, indicating that virus came by way of blood from testicles
T Temperature

TEXT-FIG. 1. Outline of the method pursued in the study of Virus III. Rabbit 9 was inoculated in the testicles in the usual manner. 5 days after the inoculation, however, the virus was recovered from the blood of this animal and demonstrated by testicular passages through several rabbits.

of M. I., from which Virus III was recovered, will be presented and discussed in detail (Text-fig. 1). It was in the fourth rabbit of the series that a reaction was first noticed, in the eye, skin, and testicles,

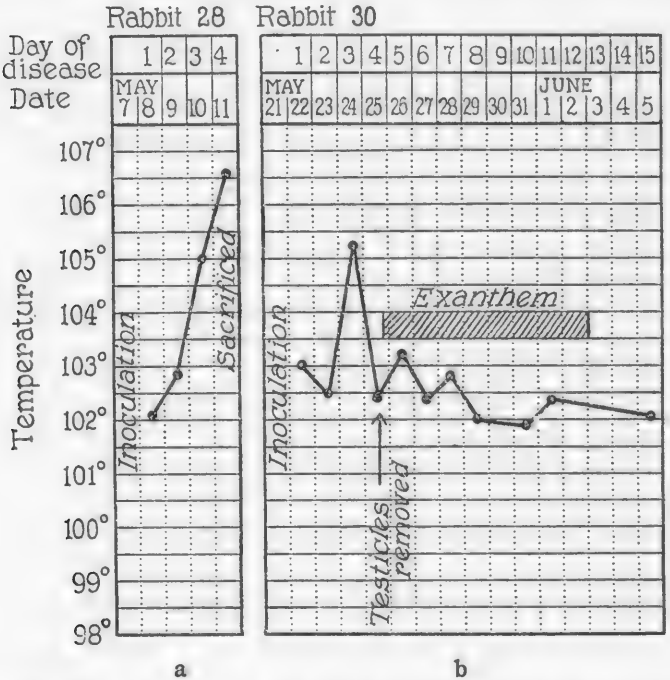
more severe than that usually caused by the trauma of inoculation. This reaction became more evident with each passage until the seventh, when a confluent eruption was obtained in the skin of Rabbit 24.



TEXT-FIG. 2, *a* and *b*. (*a*) The rabbit was inoculated in the testicles with Virus III. The chart shows the type of temperature reaction, the time of appearance and the duration of exanthem. (*b*) The rabbit was inoculated in the testicles and on the skin with Virus III which was recovered from the blood of Rabbit 9. The chart shows the type of temperature reaction, the time of appearance and the duration of take on inoculated skin. Coincident with the second rise in temperature, red papules appeared in the uninoculated shaved skin.

The virus was still alive in the testicular emulsion from Rabbit 1, after preservation for 6 days on ice in physiological salt solution as inoculation of it showed (Text-fig. 1). A whole testicle from Rabbit 4 was stored 29 days on ice in 50 per cent glycerol. Then it was

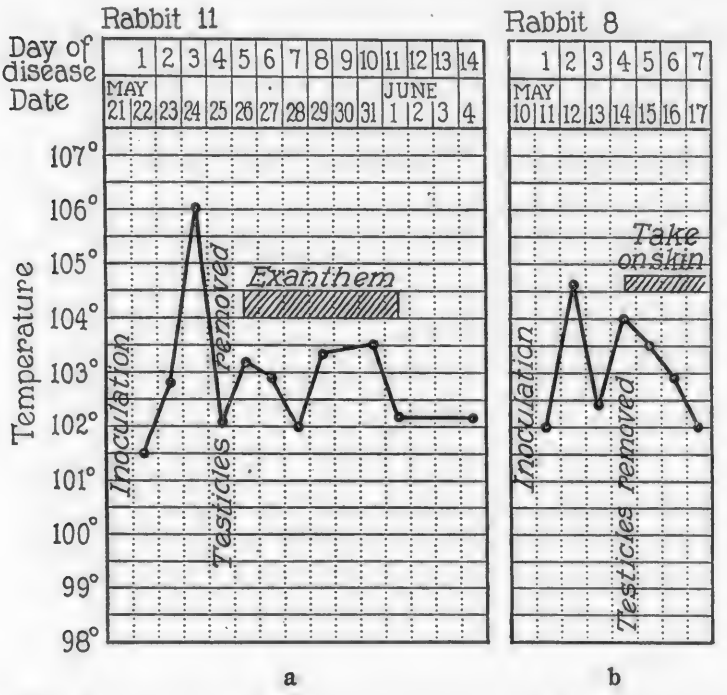
ground up and passed through rabbits in the usual way, and while the virus was still active, its virulence had decreased to such an extent that three testicular passages were necessary before it caused a



TEXT-FIG. 3, *a* and *b*. (*a*) The rabbit was inoculated in the testicles and on the skin with Virus III. The chart shows the type of temperature reaction. The animal was killed and autopsied 4 days after the inoculation. Nothing except the testicular lesion was found to account for the fever. (*b*) The rabbit was inoculated in the testicles and on the skin with Virus III. The chart shows the type of temperature reaction, the time of appearance and the duration of the exanthem which appeared in the uninoculated shaved skin.

visible reaction in the skin. The virus from Rabbit 24 also showed a decrease in virulence after it had been on ice 17 days in 50 per cent glycerol. The virus from Rabbit 28, preserved on ice 7 days in glycerol, required only one passage to revive its virulence. From these

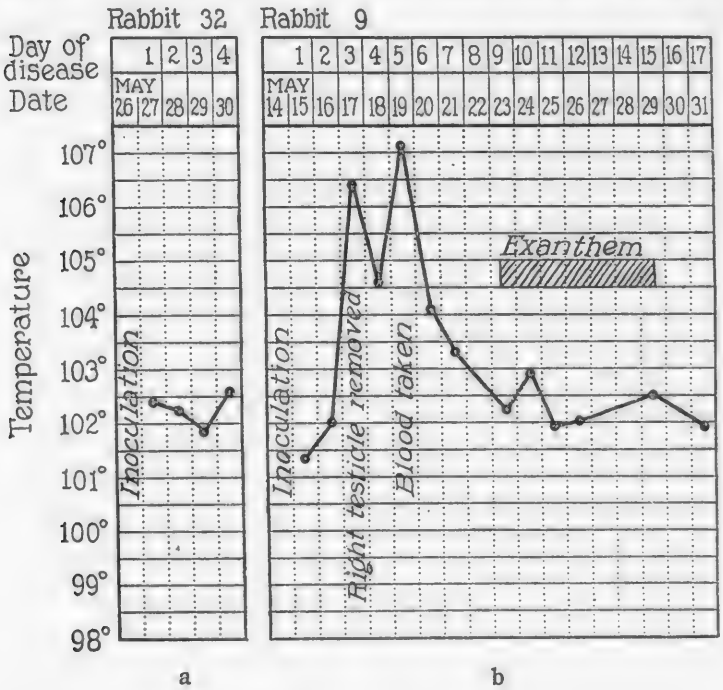
observations it is evident that Virus III survived at least for 6 days in physiological salt solution and for 29 days in 50 per cent glycerol at ice box temperature. Its virulence was decreased, but by a few passages through rabbits this was usually restored.



TEXT-FIG. 4, *a* and *b*. The rabbits were inoculated in the testicles and on the skin with Virus III. The charts show the type of temperature reaction, the time of appearance and the duration of skin take and exanthem.

Scarified corneas inoculated with Virus III showed a reaction which was not present in the controls. The reaction was not so severe as that caused by vaccine virus or the virus of herpes. It was noticeable in 3 or 4 days, and persisted for 4 to 5 days. The corneas became rough and opaque, especially along the lines of scarification. There were increased lacrimation, photophobia, and injection of

the blood vessels around the cornea. The lesions healed rapidly without permanent injury to the eye unless there was a secondary infection. A reaction in the eye occurred less constantly than in the skin. In sections of the inoculated cornea there was found along



TEXT-FIG. 5, *a* and *b*. (*a*) The usual temperature reaction in animals inoculated with a testicular emulsion free from virus. (*b*) Chart of temperature reaction and time of appearance and duration of exanthem in a rabbit from whose blood the virus was recovered. The animal was inoculated in the testicles with Virus III.

the lines of scarification a type of degeneration involving the nucleus and cytoplasm of the cells similar to the cellular changes found in chicken-pox lesions in the human skin. Occasionally small vesicles were seen in the thick layer of epithelial cells filling the defect in the cornea produced by the scarification.

Discrete lesions, following the inoculation of Virus III on the scarified skin, were small, superficial, red papules which might easily be overlooked. When the virus was concentrated enough to cause confluent lesions along the scarifications, however, the reaction was not so likely to be missed (Fig. 6). The reaction caused by the scarifications in the control skin disappeared by the 3rd or 4th day, while in the inoculated skin the erythema and the swelling of the tissues became more marked at this time, lasted 3 to 6 days longer, and then rapidly disappeared without leaving any scars. Positive results were not always obtained in the skin, even when there were reasons to justify a belief that the virus was present in the material used for the inoculation. Success seemed dependent in part at least upon the condition of the rabbits, the texture of the skin, and the depth of the scarifications. The best results were obtained when the rabbits were healthy and fat, when the skin was rather thick and firm, when the scarifications were deep, but not quite deep enough to cause bleeding, and when the virus was rubbed in thoroughly.

The pathological changes in the testicles were disregarded at first because of the amount of material injected each time. Large quantities were necessary to adapt the virus to the animals. Later, however, when the virus was more concentrated, small quantities of the supernatant fluid from thoroughly centrifuged testicular emulsions were injected. Even after these small injections the testicles would become swollen and tense in 3 to 4 days. When the testicles were removed for examination, or for passage to another animal, they were red, swollen or edematous, with whitish areas scattered over the surface. Upon sectioning them the swollen condition became more evident, as the parenchyma bulged out from the restraining capsule. This reaction is similar to, but less severe than, that caused by vaccine virus.

After the virus became adapted to rabbits, a general reaction was often observed in addition to the local ones in the eye, skin, and testicles inoculated. The rabbits looked sick, refused to eat, lost weight, occasionally developed a diarrhea, and at times had temperatures of 104–107° (Text-figs. 1 to 5). It is true that the temperatures of rabbits vary a great deal, but, as a rule, even in rabbits a temperature above 104° is pathological and one of 105–107° certainly is. The

rabbits did not always have fever, even when marked local reactions occurred in the eye, skin, and testicles. On the other hand, in some of the animals which had high temperatures, discrete lesions appeared in the control areas of the skin or in the shaved skin of rabbits inoculated only in the testicles (Fig. 7). These lesions appeared 5 to 11 days after the inoculation, endured 3 to 7 days, and disappeared without scarring (Text-figs. 1 to 5). They were red macules and papules of various sizes. Sometimes a zone of erythema, less intense than that of the papules, surrounded each lesion. The intensity of the erythema depended a good deal on the color and texture of the rabbit's skin. Coincident with the healing of the lesions, scales formed, and a brownish pigmentation of the skin appeared at these points and persisted for a number of days.

Two types of lesions were observed in the skin of rabbits as the result of shaving alone. One of these was a diffuse or macular erythema, most evident 24 to 48 hours after the shaving, disappearing rapidly and not visible after 4 to 5 days; the other consisted of small whitish nodules of uniform size without redness in or around them, appearing 10 to 14 days after the shaving and usually remaining for a long time. In contrast to the non-specific lesions, certain other lesions occurred in the uninoculated shaved skin of rabbits into the testicles of which Virus III was injected. These lesions, red macules and papules, appeared 5 to 11 days after the inoculation, disappeared rapidly, and were interpreted by us as the result of a general infection with the virus. Whether or not the discrete lesions represent a specific reaction on the part of the skin to a virus carried there by the blood, there can be no doubt that the virus has been recovered from the heart's blood of a rabbit inoculated only in the testicles (Text-figs. 1, 2, *b*, and 5, *b*).

Small pieces of skin showing the different types of lesions were removed for histological examination. A description of the pathological changes which occurred in the experimental animals and a comparison with those found in the lesions of human chicken-pox will be made in detail in another paper. In brief, however, the inoculated skin along the lines of scarification showed a cellular infiltration of the corium, a swelling and thickening of the epidermis, and certain intracellular changes similar to those observed in the papular lesions of human chicken-pox.

It proved impossible to predict with regularity whether a visible reaction would occur in any individual rabbit even when a concentrated virus was smeared on the scarified skin. This, to a certain extent, interfered with the demonstration of an immunity in recovered animals and with the determination of the effect of various temperatures on the virus. Work on these points is still in progress and will be reported later. Evidence has been obtained, however, that the virus is destroyed at a temperature of 70°C. for 30 minutes; that at least three of the strains are identical; and that protection against these three strains is not secured by an immunity to vaccine virus.

DISCUSSION.

A great deal has been written about the identity of chicken-pox and herpes zoster. Parounagian and Goodman (42) believe that some of the cases of varicella and herpes zoster reported as occurring simultaneously in the same patient are only instances of generalized herpes zoster. From observations given in the present paper it seems at least possible that certain of the cases may be instances of the localization of the virus of varicella in areas of irritated skin in such a way as to produce zoster-like lesions. Though herpes zoster and chicken-pox may possibly be identical, convincing proof is lacking, in spite of de Lange's (43) work on complement fixation in zoster and varicella. Tièche (32) reported that irritation causes herpetiform lesions in smallpox and modified smallpox, and that such an occurrence is rare in chicken-pox. He emphasized this point in the differential diagnosis of the diseases. One should be careful, however, not to place too much dependence in such a statement, as in 10 per cent of our patients with chicken-pox irritation of the skin caused a localization of the virus in the affected areas.

In working with material supposed to contain a virus which is invisible, or not recognizable, and about which practically nothing is known experimentally, one can easily be mistaken about the identity of the virus or even in regard to its presence. In spite of these difficulties we feel warranted in saying that a virus has been recovered from patients with varicella under the conditions outlined, and that the reactions observed in rabbits are more than the results of a nonspecific irritation. Further evidence must be obtained, however,

before one can think and speak definitely of this virus as the etiological agent of varicella. The ultimate proof depends upon the type of reaction this virus will cause in man and upon the possibility of protecting against varicella by inoculations of the virus.

CONCLUSIONS.

1. The localization of the virus of varicella in the human skin is influenced by irritation. This is indirect evidence that the virus is in the blood.

2. Rabbits are susceptible to a virus recovered from the blood of varicella patients.

3. Testicular emulsions containing the virus are free from ordinary aerobic and anaerobic bacteria, and produce lesions in the cornea, skin, and testicles of rabbits.

4. The virus can be transmitted indefinitely from rabbit to rabbit by means of testicular inoculation, and can be preserved 29 days in 50 per cent glycerol at a low temperature.

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EXPLANATION OF PLATES.

PLATES 38 AND 39.

FIGS. 1 to 4. Photographs showing the effect of irritation on the localization of the virus of varicella in the human skin.

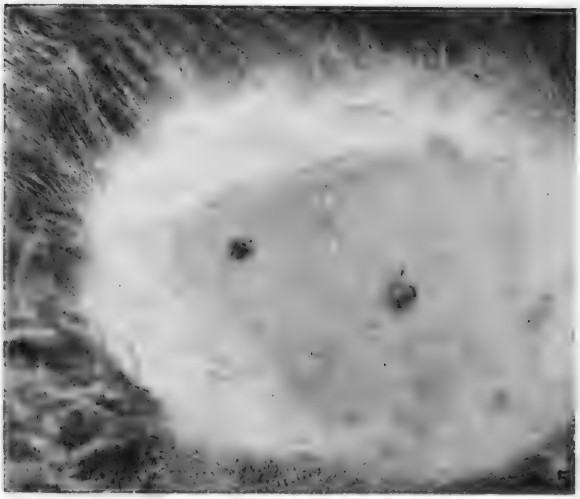
FIG. 5. Two typical vaccine pustules in the skin of a rabbit. The virus was localized in the skin by irritation after the intravenous injection of 10 cc. of blood from a rabbit vaccinated 4 days previously.

PLATE 40.

FIG. 6. (*a*) Rabbit 8. Confluent eruption along the lines of scarifications in the skin inoculated with Virus III. (*b*) Control area in the same animal. 5th day after the inoculation.

FIG. 7. Discrete papular skin lesions in Rabbit 30. The rabbit was inoculated in the testicles with Virus III. The skin was shaved and irritated immediately afterwards. The virus invaded the blood stream and the irritation caused it to localize in the shaved area. The drawing was made on the 6th day after the inoculation.

(Rivers and Tillett: Studies on varicella.)



(Rivers and Tillett: Studies on varicella.)

a. b.

FIG. 6.

FIG. 7.

(Rivers and Tillett: Studies on varicella.)

EXPERIMENTAL STUDIES ON THE ETIOLOGY OF TYPHUS FEVER.

V. Survival of the Virus in Collodion Sacs Implanted Intraabdominally in Guinea Pigs.

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In a previous paper[1] of this series, one of the writers showed that the typhus virus found in the blood of guinea pigs during the height of typical experimental typhus fever does not survive at 37°C. in anaerobic media for as long a period as in the same media under aerobic conditions. When oxygen is excluded, the viability period is 24 to 48 hours, whereas in the same media unprotected from atmospheric oxygen, the period is usually 5 days.

The virus as it exists in the tissues of guinea pigs with the experimental disease has been found to resist drying for 6 days.[2] Landsteiner and Hausmann[3] determined that frozen brain emulsions containing the virus remained active for 6 to 7 days. Nicolle and Blaizot[4] kept similar frozen material mixed with two parts of saline solution and one part of gelatin for 6 days without loss of virulence. At incubator temperature (37°C.), however, the activity of the material was lost after 2 days. Otto and Chou[5] and Otto and Papamarku[6] showed that virus in brain emulsions kept in the ice box (at +8°C.) retained its infectivity for 4 to 7 days, but the authors first mentioned found that when the material is added to normal guinea pig serum, the period of viability is lengthened to 10 days. However, at 37°C., the period is diminished to 6 days. Recently, Wolbach and his coworkers,[7]

[1] Olitsky, P. K., *J. Exp. Med.*, 1922, xxxv, 115.

[2] Olitsky, P. K., *J. Exp. Med.*, 1922, xxxv, 121.

[3] Landsteiner, K., and Hausmann, W., *Med. Klin.*, 1918, xiv, 515.

[4] Nicolle, C., and Blaizot, L., *Compt. rend. Acad.*, 1915, clxi, 646.

[5] Otto, R., and Chou, C. C., *Centr. Bakt., 1. Abt., Orig.*, 1922, lxxxviii, 467.

[6] Otto, R., and Papamarku, P., *Centr. Bakt., 1. Abt., Orig.*, 1920, lxxxiv, 12.

[7] Wolbach, S. B., Pinkerton, H., and Schlesinger, M. J., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 270.

employing tissue cultures of infected guinea pig brains, have found these cultures of the first generation to be infective up to 14 days. This is the longest period as yet recorded during which the virus has maintained its activity.

A review of the literature reveals that the typhus virus present in the brain of the guinea pigs with the experimental disease survives 6 to 7 days at freezing temperatures but when mixed with ordinary media, such as serum, under aerobic conditions, it survives for 10 days. At 37°C., the virus in the blood or brain can maintain its infectivity for 6 days; in ordinary media such as broth or serum, from 5 to 6 days; and in tissue cultures for periods up to 14 days.

In work previously reported we have employed the deductive method to study the incitant of typhus fever[1] and have demonstrated the need of the virus for oxygen. Using the method once again we have now attempted to lengthen by experimental procedures its period of survival under artificial conditions.

We sought to employ a method which would keep the virus under constant oxygen tension and at the same time give a continuous supply of nutritive elements. It is known that there is a considerable oxygen tension in the peritoneal cavity. Haggard and Henderson[8] estimated this tension at about 45 mm. Gates has shown[9] that nutrient materials from the body of the guinea pig pass through collodion sacs with sufficient rapidity to promote a luxuriant growth of bacteria, even when they are in distilled water. By employing the collodion sacs described by Gates[9] and implanting them intraperitoneally after inoculation with the typhus virus, both our requirements are met.

Method.

Collodion sacs for intraperitoneal or intraabdominal implantation were carefully prepared according to Gates' instructions.[9] Caution was exercised to make the sacs permeable, yet not so thin as to cause breakage.

4 cc. of citrated blood were obtained, as described elsewhere,[10] from guinea pigs in the 1st, 2nd, or 3rd day of the febrile period of the experimental disease. By means of a Pasteur pipette the blood was transferred to the sac, which was sealed and then implanted,[11] under the strictest asepsis, in the peritoneal cavity of a nor-

[8] Haggard, H. W., and Henderson, Y., *J. Biol. Chem.*, 1919, xxxviii, 71.

[9] Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 25.

[10] Olitsky, P. K., *J. Exp. Med.*, 1921, xxxiv, 365.

[11] All operations were performed under complete ether anesthesia.

mal, healthy, 350 gm. guinea pig. In some experiments, two inoculated sacs were placed in the same guinea pig without any apparent inconvenience to the animal. After 28 days to 6 weeks the sacs were removed from the guinea pig and the contents poured into a Petri dish or the clotted blood with which, as a rule, the sacs were tensely distended was macerated and from 2 to 3 cc. of this material were injected intraperitoneally into normal guinea pigs to test for infectivity.

In duplicate experiments, instead of employing virulent blood, the infected cerebral or splenic tissue was inoculated into the sacs. In all these instances in which tissue was used, the sacs were found broken after 1 to 2 weeks, probably as result of omosis brought about by tissue autolysis. This difficulty remains to be overcome.

Guinea pigs harboring intraperitoneally sacs inoculated with blood containing typhus virus showed in three different experiments no febrile or other reactions. When, on the other hand, the contents of sacs removed after 28 to 31 days were injected into normal guinea pigs, these developed typical experimental typhus fever, showing that the virus was still active. The criteria for determining the typical reaction were: (*a*) Transmissibility of the virus from guinea pig to guinea pig indefinitely. (*b*) Specific pathology of the affected animals during the height of the reaction. There were no apparent macroscopic changes in the organs except an enlarged spleen and a petechial rash in the skin.[12] Histological examination revealed the typical vascular and nodular changes in the different organs, especially about the blood vessels of the brain. (*c*) Absence of concomitant or secondary infections with bacteria of the ordinary species, and absence of growth on blood culture in the usual media. (*d*) The presence of cross-immunity on test with known typhus virus. All these conditions were fulfilled in our inoculated animals, as required for the definition of the experimental disease, a point emphasized by Doerr[13] and others.[1]

Guinea pigs carrying sacs inoculated with blood containing typhus virus intraabdominally for 7 weeks were, like those observed for shorter periods, unaffected throughout this time; but the sac contents tested at the end of the period failed to be infectious.

Two experiments were made in which sacs with a typhus content were implanted intraabdominally in rabbits. After 2 weeks in the animals, the sac contents proved inactive for guinea pigs.

[12] The skin lesion will be described in detail in a later paper.

[13] Doerr, R., *Centr. Bakt., I. Abt., Orig.*, 1921, lxxxv, suppl., 2.

DISCUSSION.

By implanting intraperitoneally in guinea pigs Gates' permeable sacs inoculated with blood containing typhus virus, the period of viability of the virus under artificial conditions has been extended to 31 days. This we believe is the longest time the typhus virus has been so maintained. The oxygen tension in the peritoneal cavity fulfills a necessary requirement of the virus, and the ready osmosis from the body fluids of the guinea pig into the sac undoubtedly acts to prolong viability. In this latter relation it is significant that similar experiments with the rabbit have failed. It is difficult to state at present whether an active multiplication of the virus occurs within the sac, but the prolongation of the viability of the virus therein offers ground for the hope that the method may be useful in cultivation tests.

CONCLUSION.

The typhus virus contained in the blood of guinea pigs at the height of the experimental disease remains infective for 31 days in collodion sacs placed within the abdominal cavity of guinea pigs.

INCLUSION BODIES IN EXPERIMENTAL HERPETIC INFECTION OF RABBITS.

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PLATES 41 AND 42.

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While few investigators claim to have demonstrated the actual infective agent in herpes,[1] certain peculiar nuclear and cytoplasmic inclusion bodies, which are said to be specific, continue to receive attention. Within the past 2 years it has been suggested, among other possibilities, that they are the infective agent or virus itself, "or particles of organic matter to which the virus tenaciously clings;" that they represent a combination between the virus and substances of cellular origin; that they are the expression of peculiar nucleolar changes caused by the virus; and lastly that they are merely products of cellular degeneration of dubious specificity.[2–5] The problem is more important than it might appear to be at first sight. It is not simply a question of the etiology of a relatively mild infection, with the appearance of which we are familiar, because information is accumulating to the effect that herpes may be but one of a group of diseases of unknown etiology. For instance, etiological relationship between herpes and lethargic encephalitis has been suggested.[4] Interesting inclusion bodies have likewise been described in vaccinia, chicken-pox, smallpox, and several other infections the nature of which is still under discussion.

[1] Kooy, J. M., *Klin. Monatsbl. Augenheilk.*, 1921, lxvi, 81.

[2] da Fano, C., *J. Path. and Bact.*, 1923, xxvi, 85.

[3] Lipschütz, B., *Arch. Dermatol. u. Syph.*, 1921, cxxxvi, 479.

[4] Levaditi, C., Harvier, P., and Nicolau, S., *Ann. Inst. Pasteur*, 1922, xxxvi, 77. Doerr, R., and Schnabel, A., *Z. Hyg. u. Infectionskrankh.*, 1921, xciv, 29. Levaditi, C., Ectodermoses neurotropes. Poliomyélite, encéphalite, herpès, Paris, 1922.

[5] Luger, A., and Lauda, E., *Wien. klin. Woch.*, 1921, xxxiv, 132.

As part of a wider study of the etiology of febrile herpes and of epidemic encephalitis that is being carried out by Dr. Flexner, Dr. Noguchi, and Dr. Amoss, we undertook the minute histological investigation of the inclusion bodies in question.

Infection by Intracerebral Injection of Virus.

Our first efforts were directed toward a repetition of da Fano's observations of the inclusion bodies of herpetic encephalitis in rabbits. To this end a total of eleven rabbits (Nos. 3, 5 to 9, 12, 13, 18, 21, and 30) were, in the course of our experiments, injected intracerebrally with about 0.2 cc. of 10 per cent H. F. strain of herpes virus. All showed typical symptoms at about the 4th day, when they were killed with chloroform.

Upon autopsy, sterility tests of the brain substance were made with plain broth, slant agar, and the Smith-Noguchi medium of all the rabbits except three (Nos. 3, 5, and 6). Upon incubation, the tubes remained sterile in all of these except two (Nos. 16 and 17, slant agar) in which a growth of a contaminating organism was obtained.

As a routine procedure, selected hemisections of the brain from the region of the anterior commissure, posterior commissure, and calamus scriptorius were preserved by the Orth-Müller method advised by da Fano,[6] but for some tissues the following supplementary fixatives were employed: 95 per cent alcohol, sublimate-acetic, alcoholic sublimate, 10 per cent formalin, and the mixtures of Flemming, Regaud, Carnoy, Marchi, Zenker (with and without acetic acid), and Pianese. Sections were colored by Giemsa's and Borrel's stains and, in special cases, with eosin-methylene blue, hematoxylin, fuchsin-methyl green, iron-hematoxylin, Sudan III, and other dyes.

When the plane of section passed through the lesion or in its vicinity (Nos. 3, 8, 9, 12, and 13) abundant granulations were observed resembling those described by da Fano. They were also seen in remote parts of the brain but not in such large numbers. For convenience, they may be considered under the three headings suggested by him.[7]

[6] The formula for Orth's fluid as given on page 91 of da Fano's paper is obviously a typographical error. We employed a mixture consisting of 9 parts of Müller's fluid and 1 part of commercial formalin.

[7] da Fano,[2] page 109.

1. Intranuclear Particles.—Two types of nuclear inclusions were noted: (*a*) Basophilic bodies which are probably derived from nuclear chromatin as da Fano[2] has intimated. Reference to Figs. 1 to 7 will illustrate a close topographic association between the development of these bodies and changes in the nucleolus and nuclear chromatin. But the fact that these nucleolar changes do not exactly parallel other alterations in the cells is indicated by their frequent absence in highly vacuolated cells (Figs. 8 to 11). The inclusions represented in Fig. 3 should be compared with the *neurocorpuscles encéphalitiques* of Levaditi, Harvier, and Nicolau[4] as shown in their Plate I, Fig. 1. It is interesting to note that somewhat similar nuclear inclusions have been reported in nerve cells in the vicinity of a glioma by Lafora,[8] and others, though less pronounced, in normal nerve cells by Cajal.[9] (*b*) Irregular, amorphous masses of material colored red with Giemsa's stain and exhibiting a marked affinity for eosin. These were found to be particularly abundant in the hippocampus and seem to arise through a coagulation and alteration of the nucleoplasm (Figs. 3 and 6). Sometimes acidophilic material of this kind was observed to fill the nucleus almost completely, often giving it a swollen appearance so that the remaining chromatin was restricted to a thin layer just beneath the nuclear membrane (Fig. 27). These masses resemble nuclear inclusions previously described by many investigators including Lipschütz,[3] da Fano,[2] and Goodpasture and Teague.[10]

2. Relatively Large Extra- and Intracellular Formations.—Larger granulations corresponding to da Fano's descriptions were observed in abundance. Although a few were found within nerve and neuroglia cells, as well as in endothelial cells and polymorphonuclear leucocytes, the majority occurred in certain large mononuclear cells, which vital staining with trypan blue showed to be of the type of macrophages. The granules were always spherical and were optically of homogeneous consistency. They varied in diameter from about 0.6 to 5 microns (Figs. 22 to 31). The larger ones were very resistant

[8] Lafora, G. R., *Trab. lab. invest. biol. Univ. Madrid*, 1916, xiv, 103, Fig. 2.

[9] Cajal, S. R., *Trab. lab. invest. biol. Univ. Madrid*, 1910, viii, 52.

[10] Goodpasture, E. W., and Teague, O., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 400.

to solvent action, appearing after all the fixatives enumerated on page 696. They stained blue with Giemsa, red with Borrel, and were strongly fuchsinophilic. They were blackened by the osmic acid contained in Marchi's fluid and Flemming's fluid and in a special mixture consisting of osmic, formic, and chromic acid, formalin, potassium bichromate, and chloride, and platinum chloride. They stained feebly with Sudan III, but gave a marked reaction when treated by Macallum's technique for the demonstration of masked iron. They resemble the inclusions which we have elsewhere described in vaccinia[11] in that they may be preserved by similar fixatives and that they exhibit an equally marked affinity for Borrel's stain which, we may note, was originally designed for the specific coloration of the corpuscles of Guarnieri; but they differ from the corpuscles of Guarnieri chiefly in being usually of smaller size, in their failure to indent conspicuously the nuclei of the cells containing them, and by the circumstance that they are not made up of several materials of different staining reaction but become colored uniformly throughout their substance.

That they are not readily autolyzed was shown by the following experiment. When Rabbit 3 showed typical symptoms of herpetic encephalitis, it was chloroformed and pieces of the brain were fixed in Orth's and Regaud's fluids. The remainder was placed in 50 per cent glycerol in the refrigerator at 4°C. Other pieces were preserved in the same way at 3 succeeding weekly intervals and sections were stained by the methods of Giemsa and Borrel. No reduction was noted in the large inclusion bodies, nor did they show a change in staining reaction. 9 weeks later the infectivity of the remaining material was proved (No. 30) and it is known that infectivity persists considerably longer.

Da Fano[2] has expressed the belief that these relatively large granules "originate from the progressive fragmentation of the nuclei of various elements, but chiefly of the polymorphonuclear leucocytes, under the influence of the virus." But the stains used by da Fano, *i.e.* toluidine blue, thionine, Azur II, methylene blue, and polychrome methylene blue, reveal only a fraction of the granules actually pres-

[11] Cowdry, E. V., *J. Exp. Med.*, 1922, xxxvi, 667.

ent in the tissues. His fixation in Orth's mixture followed by Müller's fluid is an excellent preservative for mitochondria, which may be brought to light in addition to the other granulations, seen in Giemsa preparations, by staining in the usual way with fuchsin-methyl green. Specimens of this kind exhibit a bewildering array of granules of different sizes, colored all tints from red to green and purple. It is not a difficult task to establish a gradation in shape and size between typical mitochondria and some of the large droplets under consideration, but it is impossible without laborious solubility tests to ascertain whether a true chemical transition between the two actually exists. It is also doubtful whether some of these larger inclusions are not stages in the phagocytosis of injured red blood corpuscles, a process recently emphasized by Woodcock.[12]

For the present, therefore, we have to entertain the possibility that the granules in question are nuclear detritus, as suggested by da Fano, or that they are formed from mitochondria, or through the phagocytosis of erythrocytes, or by some method of which we have no knowledge whatsoever. More accurate analysis might even show that all of these "relatively large" granules do not belong to the same category. Nor can we evade the corollary that we have no good reason to suppose that they are all formed in one way, in spite of the fact that they may look superficially alike. Methods of chemical analysis are hazardous when projected into microscopic fields and are only helpful when we recognize the full extent of their fallibility.

Fortunately the exact nature of the granules is not of great moment in the etiology of herpes, because, although we have failed to find them in the brains of three supposedly normal rabbits (Nos. 28, 29, and 36) we have seen granules closely resembling them in the brain lesions of typhus fever in guinea pigs, and a survey of the literature reveals the fact that inclusions of similar morphology, though perhaps of different chemical constitution, have been reported in *moquillo*, a catarrhal infection of dogs.[13]

[12] Woodcock, H. M., *J. Roy. Army Med. Corps*, 1922, xxxix, 14, and earlier papers.

[13] del Rio Hortega, P., *Trab. lab. invest. biol. Univ. Madrid*, 1915, xii, 109, Fig. 7.

But in order to have a definite answer as to whether the granules are inseparable from the infection, three rabbits (Nos. 10, 11, and 14) were injected intracerebrally with a suspension of normal brain substance, instead of brain containing the virus, by means of exactly the same technique. They were chloroformed 7, 24, and 48 hours later and the tissues at the site of inoculation were, in each case, preserved in 10 per cent formalin and stained by the methods of Giemsa and Borrel. Sections from the first and the last mentioned animals contained a few relatively large inclusions (Fig. 31), apparently identical with those under consideration, but none were seen in the second rabbit.

An additional control is supplied by Merzbacher[14] who, some years ago, made a study of traumatic encephalitis in rabbits. He has reported and clearly illustrated (Plate II, Fig. 4) a series of granulations which blacken with osmic acid, like those under discussion, and which, in their morphology, suggest some of the inclusions described by da Fano and represented in his Figs. 20 and 21. The account given by Homén[15] of infective toxic encephalitis has also proved helpful.

3. Minute Bodies.—Very small bodies of rather irregular size were met with in larger or smaller numbers in all the brains infected by this intracerebral route (Figs. 11 to 20). Some of them closely resembled the "minute bodies" of da Fano as illustrated, for example, in his Fig. 21. In this drawing it will be noted that the bodies are subject to a relatively great variation in size, some being five times as large as others. This variability is even more marked in his Fig. 24. Smears from one brain (No. 18) were dried in air, stained with Giemsa, and carefully examined. They contained large numbers of very small bodies of coccal (0.34 micron), diplococcal, and bacillary form (0.34 by 0.5 micron) stained deep blue which were often surrounded by halos and were very much like the most uniform minute bodies mentioned by da Fano. These bodies (Figs. 11 to 20) were found to be Gram-negative and to occur chiefly in neuroglia cells, but also in nerve cells, in endothelial cells, in macrophages, and extracellularly. Some were intranuclear as illustrated in Figs. 15 and 17 to 19. They

[14] Merzbacher, L., *Histol. u. histopath. Arb.*, 1909–10, iii, 139.
[15] Homén, E. A., *Arb. path. Inst. Univ. Helsingfors*, 1919, ii, 1.

are somewhat smaller than the diplococci described by Rosenow and Jackson[16] in lethargic encephalitis and are present in much greater numbers, but resemble slightly some granules illustrated by Pollak[17] in nerve cells in puerperal eclampsia. They were not observed in tissues which had been allowed to undergo partial autolysis. In Marchi and Flemming preparations of No. 30 bodies of approximately the same size and distribution were blackened with osmic acid but they may or may not be the same as those with which we have to deal because the osmic reaction cannot be superposed upon Giemsa specimens.

With a view to investigating further the significance of these bodies under more favorable conditions, we directed our attention to a close examination of the brains of rabbits infected by other routes than intracerebral injection, thus obviating all mechanical injury. In this connection it is to be recalled that da Fano made a similar control by the examination of one rabbit inoculated intranasally in which he succeeded in finding the same minute bodies which he observed following intracerebral inoculation.

Infection by Intraperitoneal and Subcutaneous Injection, by Skin Scarification, by Nasal Implantation, and by Corneal Inoculation.

The animals employed for study exhibited typical symptoms with considerable regularity in about 10 days time. The same bacteriological tests were made and turned out to be negative. Twenty-two rabbits were thus employed, as follows:

4 infected by intraperitoneal injection (Nos. 20, 23, 37, and 38).
7 infected by subcutaneous injection (Nos. 15, 19, 22, 31 to 33, and 40).
4 infected by skin scarification (Nos. 24, 26, 27, and 39).
3 infected by nasal implantation (Nos. 16, 17, and 25).
4 infected by corneal inoculation (Nos. 1, 2, 4, and 41).

In several brains the same intranuclear and relatively large granules were observed, which, as we have shown, cannot be considered to play the part of causative agents, so that henceforth it was the

[16] Rosenow, E. C., and Jackson, G. H., Jr., *J. Infect. Dis.*, 1923, xxxii, 144.
[17] Pollak, E., *Arb. neurol. Inst. Wien. Univ.*, 1906, xiii, 1, Fig. 15.

minute bodies which engaged all our attention. These were found in rapid succession in smears of six brains (Nos. 16 to 21) as well as in sections of two others (Nos. 23 and 27). They were not observed in similar brain smears of three guinea pigs employed in typhus fever experiments but they were detected in two of the three normal control rabbits (Nos. 28, 29, and 36) and in one rabbit which succumbed to septicemia but never showed symptoms suggestive of herpetic encephalitis (No. 27), but they were not seen in six other experimental septicemia rabbits. Not only were these minute bodies intensely stained in Giemsa preparations but they looked almost black and gave a peculiar impression of hardness. It was not found possible to identify them, with certainty, in living tissues stained supravitally with methyl green (W. T. M.), brilliant cresyl blue 2 B, Janus green (M. L. B), neutral red, and Nile blue sulfate or to ascertain their solubility in 0.1 per cent solutions of HCl and KOH. Some of their reactions were tested by the method of superposition, as follows: Certain of the minute bodies were identified in Giemsa preparations by a camera lucida drawing and mechanical stage readings. The preparation was then decolorized and restained by the Borrel combination and it was found that the same granules exhibit a strong affinity for magenta. Bodies similarly identified in a Giemsa preparation were decolorized and tested by the Macallum hematoxylin method for masked iron. Their reaction was negative. Proceeding in this way it was discovered that they resist solution when treated for 5 minutes with concentrated HCl, from which it may be inferred that they are not composed of chromatin. Our next task was to determine whether these minute granules have any natural coloration. Granules identified in Giemsa preparations were destained with 1 per cent potassium permanganate followed by 5 per cent oxalic acid, dehydrated, mounted, and reexamined and found to be colorless. On the chance that, in this case, the granules might have been altered through oxidation, another clump of granules was decolorized in 50 per cent alcohol which removed all the blue stain and left them a light brown color. From this we concluded that some of the minute bodies are pigments, perhaps of the type described by del Rio Hortega[18] in neuroglia cells

[18] del Rio Hortega, P., *Trab. lab. invest. biol. Univ. Madrid*, 1916, xiv, 19, Fig. 8.

using the method of Achúcarro, notwithstanding the fact that they are to be found in albinos (No. 27). But obviously it would be unsafe to state that all of the minute bodies are pigments. Some of them have certain features in common with the "methylene blue granules" illustrated by Alzheimer,[19] particularly in Plate XXVIII, which should be compared with some of da Fano's drawings and with our Figs. 11 to 13 and 20. In further effort to check our results by reference to minute bodies which may be of similar nature in conditions other than herpes, mention should also be made of the tiny and remarkably uniform granules discovered by del Rio Hortega[18] in a case of softening (of the brain) by the use of special silver methods, which, in turn, closely approximate to others described by Havet.[20] While we feel that many of the minute bodies which have been reported in the central nervous system in herpetic encephalitis are of neuroglial origin, a similar interpretation is less plausible for inclusions in other tissues devoid of neuroglia, although every organ contains fixed and wandering connective tissue elements possessing granulations which may not be so very different from those to be found in neuroglia cells.

The Cornea, Skin, Liver, Suprarenal, and Pancreas of Herpetic Rabbits.

1. Corneæ.—We examined the corneæ of the four rabbits (Nos. 1, 2, 4, and 41) mentioned on page 701, and of two others (Nos. 16 and 17) which developed corneal vesicles following nasal implantation of the virus. For comparison with the normal, we used corneal preparations made in connection with our study of vaccinia. Granules like those described by da Fano were readily found (Fig. 21). They did not differ noticeably from those which we have described in the central nervous system. In their morphology some of them call to mind the so called Russell bodies of plasma cells recently described by Dubreuil and Favre.[21] Minute bodies of pigment were

[19] Alzheimer, A., *Histol. u. histopath. Arb.*, 1909–10, iii, 555, Plate XXVIII, particularly Figs. 9 to 14.

[20] Havet, J., *Trab. lab. invest. biol. Univ. Madrid*, 1916, xiv, 74, Fig. 24; 76, Fig. 27.

[21] Dubreuil, G., and Favre, M., *Compt. rend. Soc. biol.*, 1914, lxxvii, 372.

not observed in the corneæ, but were found to be present in the neighboring skin of the eyelid (No. 16).

2. Liver, Suprarenal, and Pancreas.—About 0.3 cc. of 10 per cent virus was injected into the liver, suprarenal, and pancreas of two etherized rabbits. The inoculated areas were excised in one rabbit killed with chloroform (No. 35) 7 hours later, since, according to Lipschütz[3] the intranuclear inclusions in the cornea are best developed at this time, and, in the other (No. 34), after 24 hours as suggested by Goodpasture and Teague.[10] The tissues were fixed in Zenker, with and without acetic acid, and in Carnoy's fluid. Similar tissues from a control rabbit (No. 36) were preserved in 10 per cent formalin. Sections were colored with Giemsa's stain and hematoxylin and eosin. The only change consisted of a slight accumulation of leucocytes and the presence of a few granules of different sizes in the blood cells and connective tissue elements. Careful search revealed the presence of a few intranuclear inclusions which, however, did not seem to us to be specific to the herpetic infection.

CONCLUSIONS.

We have thus far failed to observe any inclusion bodies in herpetic lesions which in our opinion may properly be interpreted as microorganisms. Concerning the exact nature of the granules so fully reported by others before us, we hesitate to commit ourselves other than to indicate certain points of resemblance to nuclear débris, red blood cells in the process of phagocytosis, mitochondria, pigment, the "methylene blue granules" of neuroglia cells, and the Russell bodies of plasma cells. Obviously, the fact that the individual granules in a section stain in the same way by Giemsa's method, or the technique of Borrel, and look alike is no good reason to suppose that they are of the same composition. We suspect that several of our methods of staining depend more upon physical forces than upon the actual chemical constitution of the substances which become colored. So that granules which stain alike may not only differ in composition but may also have been recruited from different sources. This fact makes inferential chemical analysis extremely difficult since it is next to impossible to superpose all our reactions upon a single granule of microscopic size. Consequently, if we apply one microchemical

test to a group of granules, another test to another group, and so on, we cannot be sure that we have been dealing throughout with the same substance or substances. It is our belief that the inclusions which are so abundant in herpetic lesions do not represent a concrete class of granulations *sui generis* but that they are of variable composition and are derived from several sources.

EXPLANATION OF PLATES.

All the drawings were made with Zeiss apochromatic objective 1.5 mm., compensating ocular 6, and camera lucida at a magnification of 1,500 diameters.

PLATE 41.

FIGS. 1 to 10. Nerve cells from the nucleus caudatus of a rabbit (No. 3) injected intracerebrally with 0.2 cc. of 10 per cent H. F. virus which showed typical symptoms 4 days later. The tissue was fixed in Regaud's fluid and stained with fuchsin-methyl green, illustrating intranuclear inclusions.

FIGS. 11 to 19. Cells and isolated nuclei observed in air-dried smears stained by Giemsa's method from the brain of Rabbit 18, which received 0.2 cc. of 10 per cent suspension of the brain of No. 3 intracerebrally and showed characteristic symptoms 4 days later. The cells and some of the nuclei contain numbers of minute inclusions, colored deep blue, which subsequent staining proved to be Gram-negative.

PLATE 42.

FIG. 20. Small capillary with endothelial and adjacent neuroglial cells in the fissura hippocampi of a rabbit (No. 23) injected intracerebrally with 0.2 cc. of a 10 per cent suspension of the brain of No. 18. The tissue was fixed in 10 per cent formalin and treated with Giemsa's stain. This colored many minute inclusions deep blue, some of which were observed free within the lumen.

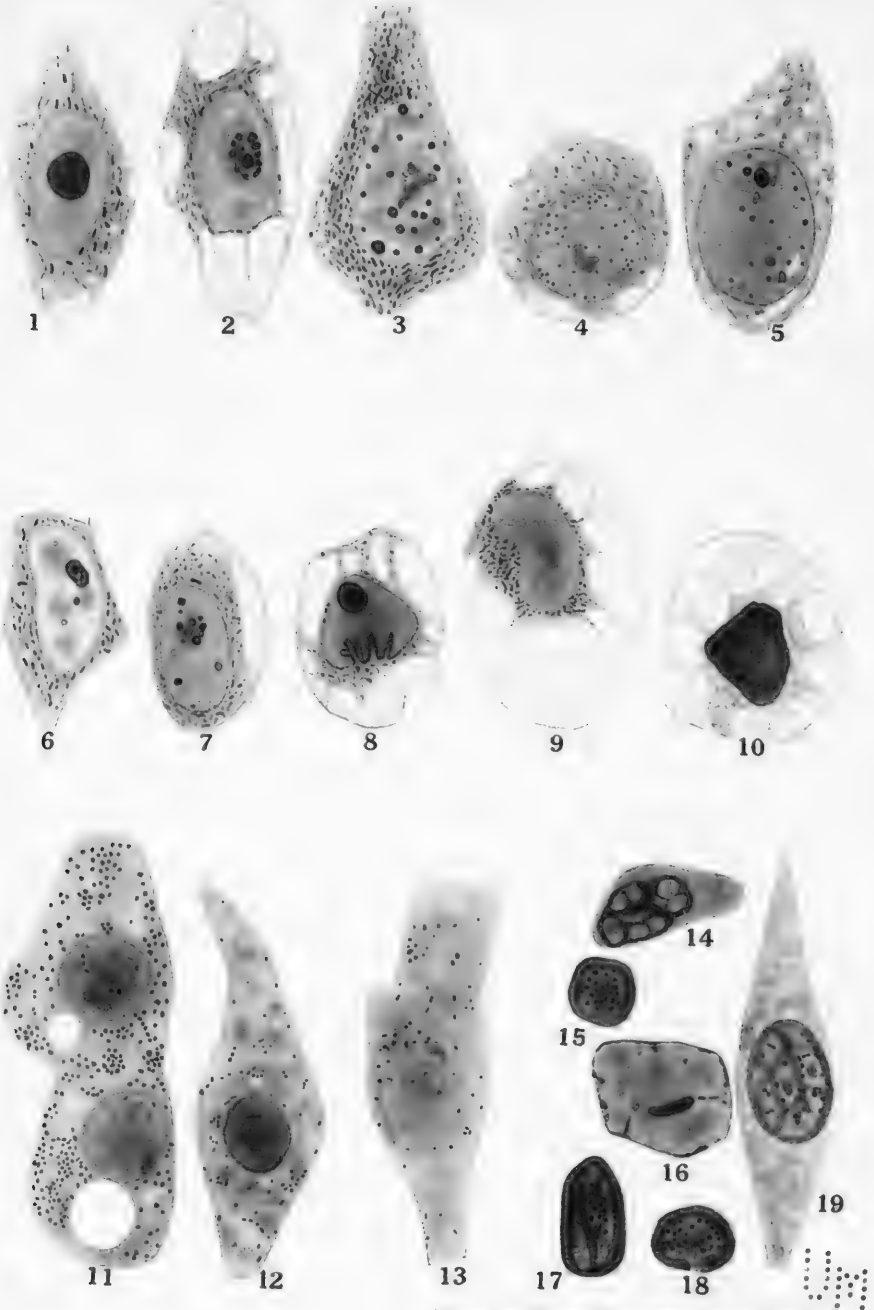
FIG. 21. A clump of phagocytes in the cornea of a rabbit (No. 1) infected by direct implantation with the virus, fixed in Zenker's fluid and colored by Giemsa's method. They contain somewhat similar "minute bodies" colored dark blue and sometimes surrounded by halos.

FIG. 22. Leucocytes and large extracellular bodies in the fascia dentata of Rabbit 3.

FIGS. 23 to 26. Selected macrophages from the meninges of Rabbit 30 which was injected intracerebrally with the brain suspension of No. 3, fixed in Flemming's fluid and stained by Giemsa's method. They contain a red blood cell and pigment (Fig. 23), leucocytes (Fig. 24), fat droplets (Fig. 25), and amorphous protein (Fig. 26).

FIG. 27. The nuclei of two nerve cells, and two leucocytes, with many inclusions in the stratum radiatum of the hippocampus of Rabbit 3 after Regaud fixation and Giemsa staining.

FIGS. 28 to 31. Selected cortical cells of a rabbit injected intracerebrally with a suspension of normal brain substance by means of exactly the same technique. Tissues taken 7 hours later were fixed in 10 per cent formalin and stained by the method of Giemsa. A variety of inclusions was observed in nerve cells (Fig. 28), in neuroglia (Figs. 29 and 30), and in certain phagocytes (Fig. 31).



(Cowdry and Nicholson: Experimental herpetic infection.)

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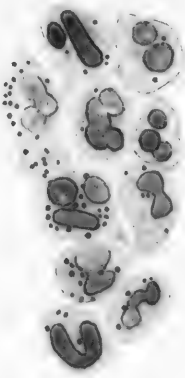

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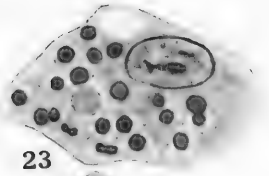

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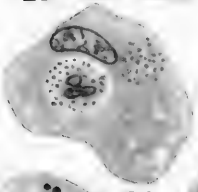

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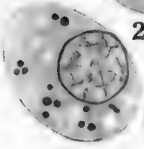

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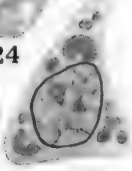

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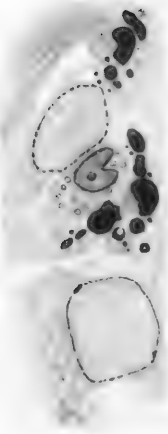

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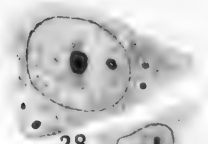

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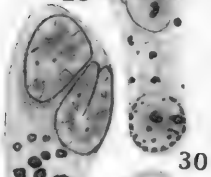

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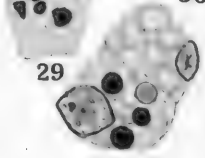

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(Cowdry and Nicholson: Experimental herpetic infection.)

A STUDY OF THE FACTORS UNDERLYING THE FORMATION OF ALVEOLAR PORES IN PNEUMONIA.

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PLATES 43 AND 44.

(Received for publication, July 19, 1923.)

While working on the reticulum of early pulmonary tuberculosis (1) several blocks of lung were received from a case of acute lobar pneumonia. Sections from these blocks show many interesting features bearing on the question of the "pores" of Kohn (2), and Hansemann (3). That these pores are not normal, preformed structures has always been the opinion expressed by the author and the study of the question from the pathological side has strengthened his previous belief.

In a previous communication on the structure of the lung (4) attention was called to the fact that "Henle describes communications between adjoining air cells, but he considers them anomalies. He found them in the lungs of old people, and attributed them to the result of atrophy and absorption of the lung substance." He was not, however, the first to describe these openings; had he added to the above description, overdistention and inflammation, he would have covered the causes which give rise to the openings. It is not improbable that phagocytes on occasion may pass through the walls of the alveoli and in this manner give rise to a pore which is only temporary in nature and structure.

The chief difficulty in understanding how these openings arise is due to a lack of knowledge of the histological structure of the alveolar walls. This is much more complex than the usual description seems to indicate.

It is not the purpose of the present contribution to go into the full details of the structure of the alveolar walls; it will suffice to say that

they consist of a network of capillary blood vessels, supported by a rich network of reticulum; a less pronounced network of elastic fibers; an occasional strand of collagenous fibers; the whole being covered on each side by a layer of epithelium. The important fact to be kept in mind is that the structures underlying the epithelium form networks with meshes which are more or less open, and that the epithelium is continuous.

In acute lobar pneumonia one of the earliest changes is an inflammatory engorgement in which the capillaries situated within the alveolar walls are distended with blood. This is followed by a serous exudate into the lumen of the alveoli together with leucocytes and red blood corpuscles. The ferments derived from the injured epithelial cells and leucocytes soon convert the fibrinogen of the blood serum into fibrin and the alveoli are now occupied by the classic exudate of red hepatization.

What concerns us at the present time is not the character of the exudate, but the injury which the pouring out of the serous and cellular exudate causes to the alveolar walls. These walls, as has been stated, have for their center a network of capillary blood vessels; outside this network of capillaries, on each side, there is a network of exquisitely fine fibrils (the reticulum), and outside this reticulum a layer of epithelial cells which in the normal, fully distended lung, have been stretched out into thin squames. Due to the pouring out of the serous exudate into this restricted space, the epithelium is pushed off and the fibrils of reticulum may, at times, give way.

Fig. 1 is a photomicrograph of a section from a case of acute lobar pneumonia. On the right of the alveolar wall, which is situated at the left of the center of the figure, two epithelial cells can be seen which have been pushed off from the alveolar wall by the accumulation of serum behind them. On the left side of the same alveolar wall, in the angle which it forms with an adjoining alveolar wall, a single cell can be seen being pushed off by the same factor. Above, and on the same side, an earlier stage in the accumulation of serum behind epithelial cells can be seen. In this section only serum is present behind the epithelial cells, although a small amount of fibrin is present within the alveolus.

Fig. 2 presents quite a different picture. Here an alveolus is seen situated between a radicle of the pulmonary vein and a bronchiole. In the upper part of the alveolus there are four cells which have a serous exudate behind them. Diagonally opposite to these cells are three cells which have been pushed off from the alveolar wall and their lower attachment to the alveolar wall has been broken. The central portion of the alveolus is filled with serum; but behind the three cells which are partially free from the alveolar wall a well marked network of fibrin can be seen.

The preceding figures have been taken from a case of lobar pneumonia in which inflammation was the factor leading to the exudation of serum and its consequent effect on the epithelium. In order to show that it is the accumulation of serum behind the epithelium which leads to its being shed off, blocks of lung from a case of mitral stenosis and insufficiency were obtained.[1] In this instance it was a mechanical, rather than an inflammatory distention of the capillaries, which occasioned the exudation.

Fig. 3 shows part of an alveolus filled with a serous exudate and along its upper border a row of epithelial cells can be seen which have been pushed off by the accumulation of serum behind them. On the opposite side a portion of the thin, expanded part of a cell can be seen raised in the form of a bleb.

Fig. 4 is from the same lung as Fig. 3 and shows some of the various shapes the alveolar epithelium assumes after being shed off and macerated for some time in the serous exudate. The study of the changes which they undergo in passing from flattened squames to large spherical cells is exceedingly interesting. Clark (5), in his study of fibrosis of the lung, evidently saw some of the early changes, for, in his figure showing a strand of "fibro-cellular" material passing through an alveolar wall,[2] he has sketched a few cells which show some of the early changes; however, he made no comment in regard to them.

Shedding of the epithelium constitutes but one step in the formation of a pore. If the epithelium on the opposite side of the alveolar wall remains intact, then no pore can result from the shedding of the

[1] The blocks were obtained through the kindness of Professor C. H. Bunting.
[2] Clark (5), p. 63, Fig. 3.

epithelium on one side of an alveolar wall. The epithelium on the opposite side of a given alveolar wall may be pushed off by the accumulation of serum behind it, but this must take place from diametrically opposite sides of an alveolar wall to play a part in the formation of a pore.

The epithelium being pushed off from diametrically opposite sides of an alveolar wall, the serum poured out from the capillaries can now find an exit into adjoining alveoli, making its way through, and possibly causing a rupture of, the delicate fibrillar network of reticulum. With the conversion of the fibrinogen into fibrin the characteristic strands passing through the alveolar walls are produced.

At times strands of fibrin can be seen radiating, cone-shaped, into an alveolus from a given point in its wall without a corresponding cone in the adjoining alveolus. In this case the epithelium of the alveolar wall in which the cone is situated has been pushed off, while that of the opposite side has remained intact. The apex of the cone corresponds to the point where the network of fibrin penetrates slightly into the alveolar wall and, possibly, also corresponds to the point where the serum found its exit. This seems the more probable because, in places where a number of adjoining cells have been pushed off, broad sheets of fibrin are frequently seen which show the same relation to the alveolar wall as the single cones of fibrin.

But what about edema? The same shedding of epithelium takes place as in lobar pneumonia, except that here the cause is mechanical and not inflammatory. Do pores exist in edema? Most assuredly they exist. If patiently looked for they can be found; but the absence of strands of fibrin to serve as guides makes them difficult to find. Moreover, in edema the epithelium is frequently completely shed off from each side of the alveolar wall; this allows the exudate to pour out freely from the entire extent of the wall.

SUMMARY.

The epithelium lining the alveolar walls is a continuous epithelium.

In both inflammatory and mechanical congestion of the pulmonary blood vessels, it is the accumulation of serum behind the epithelial cells which causes them to be shed.

The epithelium must be shed from diametrically opposite sides of an alveolar wall before a "pore" can be formed.

In both lobar pneumonia and mechanical edema pores are present, but they are not normal structures.

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EXPLANATION OF PLATES.

PLATE 43.

FIG. 1. Acute lobar pneumonia. Note the various stages in the accumulation of serum behind the epithelium seen along the alveolar wall situated to the left of the center of the figure. The exudate is almost exclusively serous, only a small quantity of fibrin is present in the alveolus. × 300.

FIG. 2. Acute lobar pneumonia. On the left of the alveolus a radicle of the pulmonary vein filled with blood. Its walls are edematous and the lymphatics are well dilated. On the right of the alveolus, part of the wall of a bronchiole. Within the alveolus, at its upper pole, a chain of four cells with a clear serous exudate behind them. Below, and to the left, three cells with a well marked network of fibrin behind them. × 300.

PLATE 44.

FIG. 3. Edema due to mitral stenosis and insufficiency. Note the chain of detached cells on the lower side of the alveolar wall and the single cell raised in the form of a bleb on the opposite side. The alveolus is filled with a well marked serous exudate. × 300.

FIG. 4. Edema due to mitral stenosis and insufficiency. The section shows edematous alveolar walls; congested blood vessels; squamous epithelial cells which are becoming more or less spherical. The longer they are macerated in the serum the larger and more spherical they become. × 300.

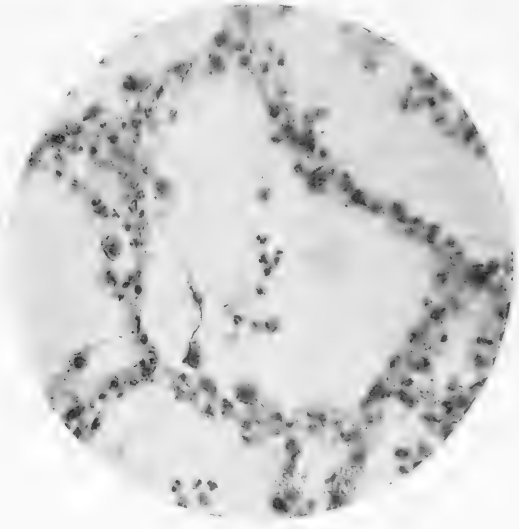

FIG. 1.

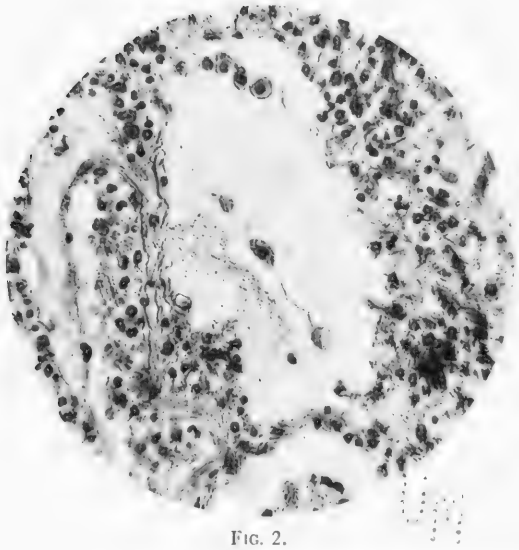

FIG. 2.

(Miller: Alveolar pores in pneumonia.)

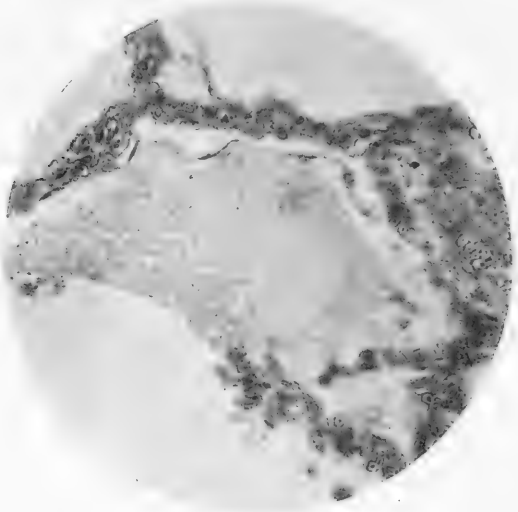

FIG. 3.

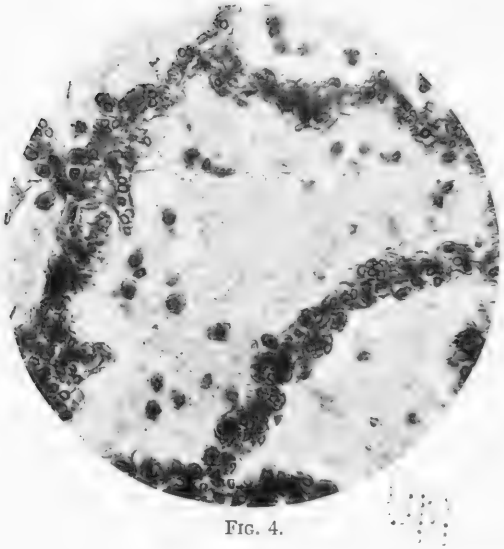

FIG. 4.

(Miller: Alveolar pores in pneumonia.)

ROENTGEN RAY INTOXICATION.

I. Bacterial Invasion of the Blood Stream as Influenced by X-Ray Destruction of the Mucosal Epithelium of the Small Intestine.

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It is a truism accepted by physiologists, bacteriologists, and pathologists alike that the covering mucosal epithelium of the intestinal tract is a very important if not the all important barrier which guards the underlying tissues, lymph, and blood vessels from overwhelming invasion by hoards of bacteria which swarm in the intestinal tract. Our experiments indicate strongly that this barrier may be wiped away completely and yet the expected overwhelming invasion does not follow. Evidently the other tissues or tissue juices have a protective mechanism, perhaps a form of secondary defense which is of much importance and is not to be neglected in estimating the resistance of the intestine to bacterial invasion. If this observation is correct this phenomenon is worthy of much careful study and should interest a variety of investigators, particularly the bacteriologist and the pathologist.

Warren and Whipple (58) have described in sufficient detail the remarkable influence of large doses of the x-ray upon the epithelium of the small intestine. Suitable doses of x-rays in normal dogs will destroy almost completely the epithelial covering of the villi and crypts of the small intestine. Such experiments present a remarkable picture in which the intestinal lumen is devoid of its epithelial lining and the naked villi and denuded crypts are covered by masses of exudate and bacteria. It is difficult to believe that under such conditions the

animal is not overwhelmed by bacterial invasion but the expected result does not follow and the inflammatory reaction is surprisingly slight.

Such experimental animals die usually on the 4th day with all clinical evidence of acute intoxication which in many respects is like that observed in intestinal obstruction. There is evidence that this x-ray intoxication is related to the epithelial cell disintegration in the mucosa but more experiments are needed to establish this point. We have observed toxic substances of proteose-like nature obtained from these injured intestines but wish to reserve a discussion of this subject for a subsequent publication.

These experiments are a continuation of the investigative program begun by Hall and Whipple (21) and extended by Warren and Whipple (58). This paper must be looked upon as a preliminary communication and the writers emphasize its incompleteness, but circumstances made it impossible for them to continue this investigation to furnish the needful quantitative measurement of bacterial invasion.

A considerable literature has accumulated in the past few years dealing with the permeability of the intestinal mucosa of various animals. The permeability of the normal and the injured mucosa has been investigated and much has been written concerning agonal and postmortem invasion of the blood stream from the intestinal tract. If space permitted it would be desirable to review some of these papers but we shall list merely the papers which have points of interest in relation to this problem.

There has been much discussion about the permeability of the normal *uninjured intestinal mucosa* to bacteria residing in the intestinal canal. Different investigators have obtained varying results with supposedly normal laboratory animals. Interesting data are to be found in papers by Trombetta (56), Metchnikoff (37), Korkunoff (32), Neisser (40), Opitz (44), Klimenko (31), Selter (46), Hornemann (28), Nocard (42), Nicolas and Descos (41), Adami (2), Ficker (15), Wrzosek (63), Holle (26), Tsunoda (57), von Wassermann and Sommerfeld (59), TenBroeck (54, 55), and Besredka (7).

The part played by intestinal injury or abnormality was investigated early in the study of invasion of the intestinal tract by bacteria. Papers dealing with intestinal incarceration, mechanical and chemical injury, parasitic injury of the intestine, and acute inflammation are reported by Oker-Blom (43), Bosc and Blanc (10), Neisser (40), Beco (5, 6), Flexner (16, 17), Birch-Hirschfeld (8), Casagrandi

(13), Arnd (3), Bosc (9), Sordoillet (49), Maklezow (35), Marfan and Bernard (36), Opitz (44), Klimenko (31), Weinberg (60), Southard and Canavan (50), C. Hess (22), O. Hess (23), Shearman and Moorhead (47), Hirst (24), and Fredette (18).

Agonal and postmortem invasion from the intestine has been carefully studied and much interesting data are to be found in papers by Trombetta (56), Beco (5, 6), Opitz (44), Klimenko (31), von Wassermann and Sommerfeld (59), Gwyn and Harris (20), Fredette (18), Southard and Canavan (12, 50), Richey and Goehring (45), Hiss and Zinsser (25), Achard and Phulpin (1), and Gay and Southard (19).

If bacteria reach the circulation their distribution in the body conforms to fairly well established mechanisms. These points are covered in papers by Ito (29), Meyer (38), Bartlett and Ozaki (4), Wyssokowitsch (64), O. Hess (23), Werigo (61, 62), Kyes (34), Bull (11), Hopkins and Parker (27), TenBroeck (54, 55), Teale and Bach (52, 53), Siebel (48), and Drinker and Shaw (14).

Latent Organisms.—In cultural studies in animals following a profound injury, there is another source for bacteria besides immediate invasion from the intestinal tract. Koser and McClelland (33) and later Teale and Bach (52, 53) pointed out that spores could be identified in the organs of animals days and weeks after injection. Teale and Bach showed further that if an animal in this state of equilibrium received an injection of almost any non-specific substance which gave a shock to the animal organism, the equilibrium would be upset often with fatal result. Perhaps some of our cultures of sporulating organisms can be explained on this basis.

Diarrhea.—Still another factor of importance which may influence bacterial invasion is the rapid and continuous emptying of the intestinal canal. Kendall (30) thinks that cathartics decrease the number of bacteria in the intestine by mechanical means. He offers no experimental data but cites Morax (39), Steiff (51), and others who studied the disinfectant action of various drugs in intestinal putrefaction.

Method.

The dogs used in these experiments were normal and healthy animals. They were killed under chloroform anesthesia by bleeding. The body was washed with lysol and immediately opened with sterile precautions. Small blocks of tissue were taken from the lungs, liver, kidney, and mesenteric lymph nodes. Unfortunately the spleen was not cultured nor were quantitative tests made. These blocks were seared and small bits from the inside were cultured in 1 per cent glucose broth for 24 hours. 25 cc. of heart's blood were put into 250 cc. of 1 per cent glucose and also cultured for 24 hours. Enrichment cultures were plated on Endo's and plain agar medium. As a rule enrichments were inoculated in beef heart media aerobically and anaerobically. *Bacillus coli* was identified by the usual sugar tests.

Smears of the intestinal contents and of the organs were made at the time of autopsy. Smears of the organs were usually negative though an occasional Gram-positive rod (*sporogenes* group) was seen. The intestinal contents showed the usual Gram-negative rods (*Bacillus coli*), enterococci, and small Gram-positive cocci and Gram-positive rods, mostly of the *sporogenes* group, though the fermentative *Bacillus welchii* was frequently found. A number of sections from the intestines, liver, and kidney of normal dogs and the radiated animals were stained with a modified Gram stain and counterstained with methylene blue for the purpose of locating and differentiating some of the organisms in these tissues. Duplicates were stained with carbolthionine. It is evident that the search for bacteria in tissues except in the case of local invasion in large numbers is apt to be futile.

EXPERIMENTAL OBSERVATIONS.

Table I represents the results of the cultural studies. *Bacillus sporogenes* was identified in the organs of the normal dog. Cocci and other organisms may also be cultured under apparently normal conditions from the mesenteric lymph nodes. Dog 19-130, sacrificed 24 hours after radiation over the abdomen, showed no qualitative evidence of invasion by other organisms. There was histological evidence of injury to the chromatin of the crypt epithelium of the small intestine[1] but there was no cell disintegration nor were there symptoms of intoxication at this time. This animal, therefore, may be looked upon as a second control. *Bacillus coli* was not found in either of these two dogs. It was not usually present except under abnormal conditions (*vide infra*).

Dog 19-101 was sacrificed 48 hours after radiation. This is the earliest period at which the first symptoms of clinical intoxication were manifest. Histologically many of the cells of the crypt epithelium were disintegrated and had fallen away from their basement membranes over a large part of the small intestine. *Bacillus coli* was cultured from the mesenteric lymph nodes, the left lung, and the right kidney. This would seem to indicate the invasion of the lymph stream, though vomiting and inspiration of this matter into

[1] Warren and Whipple, Paper II, this series.

TABLE I.

Culture of Organs of Normal and Radiated Animals.

Organs.	Dog No.					
	19–133	19–130	19–101	19–50	19–85	19–78
	Normal control.	24 hrs. after radiation.	48 hrs. after radiation.	72 hrs. after radiation.	96 hrs. after radiation.	96 hrs. after radiation (moribund).
Heart's blood.	Sterile.	Sterile.	Sterile.	Sterile.	One or two *B. sporogenes.*	*B. coli; B. sporogenes.*
Mesenteric lymph node.	Staphylococcus.	Staphylococcus.	*B. coli; Staphylococcus albus.*	*B. coli;* staphylococci; *B. sporogenes.*	*B. coli; B. welchii; B. sporogenes.*	*B. coli; B. sporogenes.*
Right lung.	A few *B. sporogenes.*	—	*B. sporogenes.*	*B. sporogenes.*	*B. welchii; B. sporogenes.*	*B. coli;* enterococci; *B. sporogenes.*
Left "	A few *B. sporogenes.*	*B. sporogenes.*	" *coli; Staphylococcus aureus.*	" "	*B. sporogenes.*	*B. coli;* cocci; *B. sporogenes.*
Right lobe of liver.	*B. sporogenes.*	" "	*B. sporogenes.*	" "	" *welchii; B. sporogenes.*	*B. sporogenes.*
Left " " "	" "	" "	" "	" "	*B. sporogenes.*	*B. coli; B. sporogenes.*
Right kidney.	" "	Sterile.	" *coli; Staphylococcus albus.*	Sterile.	" *coli; B. welchii; B. sporogenes.*	*Staphylococcus albus.*
Left "	" "	"	*B. sporogenes.*	"	*B. welchii; B. sporogenes.*	Sterile.

the lung may be responsible for the positive culture of *Bacillus coli* from the left lung. The leucocytes which were infiltrating the submucosa were not present in great numbers. They seemed concerned with the remarkable injury to the crypt epithelium rather than with bacterial invasion.

Dog 19–50 sacrificed 72 hours after radiation was much intoxicated and showed slightly greater destruction of crypt cells than Dog 19–101. The invasion of *Bacillus coli* was found only in the mesenteric lymph nodes. Otherwise the type of organisms appeared to conform to those normally present. The usual invaders may or may not have been increased in numbers but many quantitative cultures must be made to settle this point.

Of the two dogs sacrificed 96 hours after radiation, Dog 19-85 was acutely intoxicated although not yet moribund. Dog 19-78 was moribund. The mucosa of the intestine was practically destroyed in both dogs. For the first time (Dog 19-85) the heart's blood was not sterile. *Bacillus sporogenes* was found in all the organs. *Bacillus coli* was found in the mesenteric lymph nodes and the right kidney. *Bacillus welchii* was also found there and in the right lobe of the liver, the right lung, and the left kidney.

In Dog 19-78, which was sacrificed while moribund, *Bacillus coli* was found in the heart's blood as well as *Bacillus sporogenes*. *Bacillus coli* was also present in the mesenteric lymph nodes, both lungs, and the left lobe of the liver. Cocci were found in the lungs and right kidney. It is rather remarkable that the left kidney should remain sterile in the moribund dog though in the 48 hour dog and the other 96 hour dog none of the organs were sterile.

Study of the *stained slides* from the *fixed sections* showed large masses of bacteria, mostly Gram-positive rods, in the lumen of the ileum and colon and between the villi and in the crypts. Very few bacteria were seen in the duodenum. In the markedly injured sections bacteria were found among the disintegrated epithelial cells and up against the basement membrane where the epithelial cells have fallen away. At no place were any organisms found to have invaded the stroma even in the sections where the villi had collapsed and open capillaries were present. Bacteria were not observed in the few leucocytes present. Kidneys and liver when culturally positive

showed no bacteria even after prolonged search. As pointed out above, this is to be expected as only a few organisms may be present and these may be missed or they may have been washed out by the histological method.

There was no evidence of local bacterial invasion of the intestinal wall at any point. It is to be noted that *Ascaris* and tapeworms were present in all these dogs.

DISCUSSION.

It is now pretty generally accepted that the *normal mucosa* of the small intestine of the dog is actually permeable at times to the normal bacterial flora. The critic will insist that such invasion does in effect give good evidence for actual injury of the mucosa by food particles (bones, etc.). He will stress the importance of the frequent intestinal parasites of the common laboratory animals. He may further argue that much of the evidence for permeability of the normal mucosa is obtained by the use of bacteria foreign to the intestinal tract and this very procedure introduces an abnormal factor.

Actual injury of the intestinal wall gives an obvious means of bacterial invasion from the intestinal lumen. Invagination and strangulation of the gut will give not only cultural evidence of bacterial invasion within 6 to 15 hours but histological evidence of overwhelming invasion of local tissues. The natural deduction is that small injuries will likewise permit a bacterial invasion from the intestinal lumen, for example, enteritis, purgation, intestinal parasites. But we must be careful when we talk about constipation and simple catharsis and round worms as all opening the portals to bacterial invasion. This fact has not been established by any experimental data known to us and much careful *quantitative* cultural work with carefully controlled and autopsied animals will be required for the solution of this important problem. The *normal* has never been established beyond question and the very term often requires definition in various papers.

Agonal invasion of the blood stream and organs is not rare—in fact is quite common in all conditions but especially when there is abnormality of the intestinal tract. The importance of bacterial cultures in the agonal period must then be interpreted with a proper

understanding of this fact. Multiplication of organisms in the *post-mortem period* will furthur complicate a complex picture. Such postmortem observations are of limited value in a study of this type.

The heart's blood cultures in our experiments remained sterile until the last day of intoxication or until almost the agonal period. The same observation might hold for fatal respiratory distemper. It is common knowledge that many bacteria may be introduced into the blood stream without causing a bacteremia in dogs. The lungs, liver, and spleen are the important filters.

In some of the experiments of Hall and Whipple (21) the dogs survived the 4th day of intoxication and died 3 to 6 days later. Some of these dogs showed miliary kidney abscesses, liver focal necrosis, tiny infarcts in organs, and patches of pneumonia. It might be claimed that this is a result of bacterial invasion from the intestinal tract and in fact this may be the truth. But there is ground for argument to the effect that this is an invasion from the respiratory tract due to the severe intoxication, constant vomiting, and low resistance. We have found no similar lesions in this more recent series of dogs. Also a considerable series of rats and other animals exposed to x-rays failed to show abscesses or focal necroses in organs at autopsy.

We have a striking contrast in the intestinal mucosa between *excretion* or elimination or extravasation of fluid and the *absorption* following x-ray exposure. There is a *profuse excretion* throughout the intestinal tract and clinically the result is vomiting and diarrhea in spite of lack of food or fluid intake. We note escape of fluid through the mucosa, outwandering of white cells, and diapedesis, or hemorrhage of red cells. There is obvious *lack of absorption* and we note a surprising lack of bacterial invasion of the submucosa in spite of the total absence of the covering epithelium of crypts and villi. This preponderance of *flow out* through the mucosa may in fact be one of the protective factors which stand between the bacteria of the intestine and the tissues.

The mesenteric glands show evidence of a little invasion from the intestine during the 2nd, 3rd, and 4th days after x-ray exposure. It is probable that a mild enteritis would give a similar picture but here abnormalities of the mucosa would be hard to demonstrate histologically.

The outstanding feature of these experiments is the striking contrast between obvious injury of the intestinal epithelium and the lack of invasion of the intestinal bacteria into the tissues, lymph, and blood. We recall from the histology of this condition that during the 2nd and 3rd days after radiation the intestinal epithelium shows profound injury, disintegration, and separation from the basement membrane. Organ cultures at this time show almost the same picture as in control periods (Table I). During the 4th day after radiation there is severe clinical intoxication and often a preagonal coma. Organ cultures at this time show evidences of invasion of blood stream and considerable bacterial dissemination in various organs. But we might observe the same sequence in any severe intoxication leading to coma and death with complete absence of gross intestinal abnormality.

SUMMARY.

The x-ray has a specific effect upon the epithelium lining the crypts and covering the villi of the small intestine. A suitable dose of x-ray will destroy this epithelium in large measure, leaving empty crypts and naked villi exposed to swarms of bacteria in the intestine. Subsequently one does not observe an overwhelming invasion of the tissues, lymph, and blood by intestinal bacteria. It seems obvious therefore that the intestinal epithelium is not the all important barrier which protects the tissues from invasion by intestinal bacteria.

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ROENTGEN RAY INTOXICATION.

II. The Cumulative Effect or Summation of X-Ray Exposures Given at Varying Intervals.

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It is generally accepted that x-ray exposures repeated at short intervals give a summation effect but the observations are largely concerned with skin injury in human beings and little if any with the influence of the rays upon the deeper lying tissue cells. The evidence of our experiments indicates that the injury done the deep tissue cells does not follow the same summation laws observed clinically to apply to the cells of the epidermis.

We have a fairly accurate measure of the injury done to the epithelium of the small intestine and in the dog can predict with considerable accuracy the clinical reaction, lethal or sublethal, which will follow a given x-ray exposure of the abdomen. The minimum lethal dose (M.L.D.) under uniform conditions in our experiments has been pretty accurately determined and is fairly constant (Warren and Whipple (2)). Therefore we are able to give any fraction of the minimum lethal dose at long or short intervals and observe the clinical reaction, which gives us a relatively accurate measure of the amount of summation under such conditions. These clinical data are supplemented by autopsy material.

Since the completion of these experiments a paper by Kingery (1) has appeared which is of great interest in this connection. We regret that we had no opportunity to plan experiments to test more completely the interesting formula of Kingery. Our experiments as they stand indicate that Kingery's formula for x-ray skin erythema does not in any way hold for the x-ray injury of intestinal epithelium. It

is unlikely therefore that his formula can be applied to the injury done by the x-rays to cancer or tumor cells lying below the skin surface. Such evidence as we have indicates that each cell, in its own reaction to the x-ray, is a law unto itself and we have no data which enable us to predict the reaction of any given cell from knowledge concerning the reaction of any other similar or different body cell. This statement probably holds for tumor cells and the difficulty of establishing a formula to meet all requirements becomes apparent. It will be distinctly worth while to extend Kingery's observations on skin erythema to animals where more complete control can obtain. The dog is a very valuable animal for such work as its skin is quite sensitive to the x-ray and the reaction constant.

Method.

In these experiments the average *maximum sublethal dose* (320 milliampere minutes) was divided into certain fractions which were administered over the abdomen of a series of dogs at different intervals. The source of radiation was the same as that in the other experiments reported. A standard autotransformer machine was used at an E.M.F. of 85 to 95 kilovolts. The spark-gap measured 21 to 23.7 cm. between sharp points. A medium focus Coolidge tube was set at a skin target distance of 25 cm. The filament current was usually 8 milliamperes and the dosage was computed in milliampere minutes (M.A.M.). As a filter 2 mm. sheets of aluminum were used throughout. No screens were used and the abdomen was exposed in quadrants, halves, or as a whole, depending usually on the size of the animal. Complete anatomical and histological studies were made in each case at autopsy at the end of the experiment.

EXPERIMENTAL OBSERVATIONS.

Experiment 1.—Dog 20–16 was given approximately one-half of the maximum sublethal dose, *i.e.* 160 M.A.M., over a different quadrant of the abdomen at 6 day intervals. Every 2nd or 3rd day after exposure the symptoms of intoxication appeared. Intoxication increased in severity until the 4th day and then subsided. This was repeated in the same sequence with about the same severity of symptoms after each separate radiation. The total from all exposures was 640 M.A.M. (two maximal sublethal doses) within a period of 4 weeks. The dog was killed with

ether on the 34th day (16 days after the last radiation). The mucosa of the small intestine was rough and granular throughout most of its length. There were several thin walled sections where the mucosa had entirely disappeared. Microscopically there was much dead epithelium seen in every section of the small intestine. Regeneration, which occurs very rapidly, was noted everywhere in this tissue. This regeneration as is usual was made up in part of somewhat atypical cells. There was a certain amount of leucocytic infiltration at the bases of the mucosa crypts. The colon showed the same picture to a moderate degree. As a whole the picture was that of widespread destruction and injury of much of the intestinal epithelium with an effort on the part of the remaining cells to cover over the injured surfaces and regenerate toward normal. The spleen and mesenteric lymph nodes were atrophied and showed a remarkable amount of pigment and phagocytosis.

The clinical reaction, diarrhea and intoxication, was most severe following the second and third exposures. It was less intense in the later treatments. We may wish to explain this observation on the basis of increasing resistance or tolerance on the part of the intestinal epithelium or it may be that less epithelium remains following the first injury and therefore the toxic reaction resulting from subsequent injury by the x-ray is less severe. It is possible that the animal develops a tolerance for the toxic products causing the clinical reaction. According to Kingery's formula and his experimental curves it should have been safe to repeat the amount of dosage given this animal every 3½ days but from the clinical appearance of the animal we believe this procedure would have been fatal.

Experiment 2.—Dog 20–24 was given 80 M.A.M. (one-fourth of the maximal sublethal dose) over one-half the abdomen every other day. The clinical symptoms were progressively severe. Intoxication appeared at 240 to 320 M.A.M. (13th day). Death occurred 4 days later after 640 M.A.M. (two maximal sublethal doses) had been given during 14 days. There was extensive destruction of the mucosa of the jejunum and ileum with patchy areas of regeneration. Few normal epithelial cells were to be seen. Kingery's figure[1] demonstrating 25 per cent dosage on alternate days, at the end of 14 to 16 days indicates only about 50 per cent saturation but this experiment indicates a distinct oversaturation by a similar percentage dose.

Experiment 3.—Dog 20–39 was given one-fifth of the maximum sublethal dose, or 64 M.A.M., over a third of the abdomen every day for 6 days. The total, 384 M.A.M., was followed by a fatal issue 3 days after the last radiation (8 days in all). The clinical symptoms and the severity of the lesions did not differ appreciably from experiments in which the M.L.D. was given in one massive dose. The effect of the radiation was not apparent until over one-half of the total amount had been given and the severe symptoms did not start until the M.L.D. was administered. Death occurred 3 days later much as in single massive M.L.D. experiments. The destruction of the epithelium of the mucosa was widespread and showed little

[1] Kingery (1), Fig. 3.

regeneration. The anatomical picture resembled that following a M.L.D. given in a single exposure. On the other hand, Kingery's chart for 20 per cent daily dosage only reaches about 60 per cent saturation on the 5th day and by the 8th day this should have decreased to about 20 to 30 per cent or so of what he calls "residual irradiation effects."

Experiment 4.—Dog 20–28 was given one-sixth of the saturation dose, or 56 M.A.M., over a half of the abdomen every day for 6 days (a total of 336 M.A.M. within the 6 days). The severity of the symptoms increased proportionately after one-half of the dosage was administered until the sublethal amount was given, after which for several days clinical intoxication was severe. Another dose of 56 M.A.M. undoubtedly would have produced a lethal effect. The symptoms receded in much the same manner as if the sublethal dose had been given in one massive dose. The dog contracted distemper and died 13 days after the last exposure to radiation and after the symptoms following the radiation had practically subsided. There was evidence of widespread destruction of the epithelium of the small intestine with generalized epithelial cell degeneration. Roughly compared to Kingery's daily dosage chart, 15 per cent dosage at the sixth dose would have reached only 50 per cent saturation. In our experiment, however, it approached a sublethal effect by the sixth dose just as if there had been no spacing of the total dosage.

Experiment 5.—Dog 20–53 was exposed to 100 M.A.M. over a third of the abdomen every other day for three doses and 30 M.A.M. were given for the fourth dose. It was apparent here also that a full fourth dose would kill the animal. Intoxication was manifest at 200 M.A.M. and diarrhea at 300 M.A.M. The symptoms were severe and just sublethal. The animal recovered to succumb to distemper 17 days after the last radiation. Kingery's curve for 25 per cent dosage on alternate days does not pass saturation even in the 16 days charted. Our alternate daily dosage of "25 per cent" approached saturation on the 6th day. It was evident from the clinical picture that this dose was just sublethal.

DISCUSSION.

The summation of x-ray dosage is brought out very clearly by the five experiments given above. It seems that during a period of 5 or 6 days there is little difference as regards x-ray intoxication whether one large dose or a number of small doses are given, provided the same total number of milliampere minutes is administered to the dog. For example, one single dose of 320 milliampere minutes causes very severe clinical intoxication and extensive injury of the intestinal epithelium but almost always recovery follows. Again a dose of 336 milliampere minutes given in divided doses of 56 milliampere minutes every day for 6 days gives very severe sublethal intoxication and recovery (Experiment 4). The onset of the intoxication is a

little more gradual when the small doses are given but otherwise there is little or no difference. At times we may suspect that a dog will tolerate a slightly greater sum of milliampere minutes in small doses than in a single large dose but this rarely exceeds 10 per cent of the large dose.

When intervals of 6 days or longer supervene between x-ray doses we find little if any summation and at times even a suggestion of increased tolerance to subsequent x-ray exposures (Experiment 1).

We recall that the picture of clinical x-ray intoxication following radiation of the abdomen in dogs presents a remarkable parallel to the sequence of changes in the epithelium of the small intestine (Warren and Whipple (2)). Epithelial injury is evident on the 1st day after radiation and marked on the 2nd day. The 3rd day shows extensive breaking down of this injured epithelium and its removal. The 4th day marks the extreme stage of epithelial injury and this coincides with the severe clinical intoxication. Epithelial repair is in evidence on the 3rd and 4th days but becomes conspicuous on the 5th, 6th, and subsequent days. One cannot escape the belief that the epithelial disintegration is responsible in large measure for the clinical intoxication. We know that disturbance of this epithelium may cause acute and fatal intoxication (intestinal obstruction and intussusception). One may suspect that split products (proteose-like bodies) of the injured epithelium may well be concerned in the essential toxic reaction.

There is convincing evidence that the amount of clinical intoxication is proportional to the amount of intestinal mucosa injured by the x-ray rather than to the size of the x-ray dose. For example,[2] a very large dose given through a small aperture in a lead screen will destroy all the intestinal epithelium in the track of this beam of radiation, causing slight clinical intoxication, but the same dose given diffusely over the abdomen with no limiting screen would be promptly fatal with widespread destruction of the epithelium of the mucosa of the small intestine.

It is clear from the above experiments that Kingery's formula does not hold for the x-ray injury of the intestinal epithelium. We are

[2] Warren and Whipple, Paper III, this series.

inclined to believe that injury of cancer cells by the x-ray will be found to resemble more closely this injury of the sensitive intestinal mucosa, and in giving repeated therapeutic x-ray treatments of cancer, the data of our experiments should serve a useful purpose.

SUMMARY.

A single large dose of x-rays over the abdomen will cause a definite injury of the mucosa of the small intestine and the severity of the clinical intoxication seems to parallel this recognizable epithelial injury. This clinical intoxication lasts 4 to 6 days if the x-ray dose is sublethal. Subsequent doses of radiation given within this period of clinical intoxication give recognizable evidence of summation or a cumulative effect.

Small but repeated doses of radiation given within a 5 or 6 day period will cause practically the same cell injury and clinical intoxication as will a single dose representing the sum of the small doses expressed in milliampere minutes. Doses of radiation given at 6 day or longer intervals show no evidence of summation.

The reaction of this relatively sensitive intestinal epithelium to radiation may be similar to the reaction of certain deep lying tumor tissues to x-ray therapy and our experiments may give information of value to physicians concerned with x-ray or radium therapy.

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ROENTGEN RAY INTOXICATION.

III. The Path of a Beam of Hard Rays in the Living Organism.

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Plate 45.

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All roentgenologists are deeply interested in the problems which may be formulated in these few questions: Do the hard x-rays follow a straight and narrow path from the x-ray target through the tissues of the human body? Do these hard x-rays suffer from reflection or refraction and therefore influence tissues outside of this direct pathway? What factor is secondary radiation in the treatment of disease and does such secondary radiation exert a toxic effect on tissue cells or tumor cells outside of the direct pathway of the hard x-rays?

The experiments outlined below help to give an answer to the above questions. We may say that our experiments indicate that the hard x-rays which injure the epithelial cells of the intestine travel in straight lines through the host's tissues with no evidence that these rays are deviated to injure tissues outside of the direct x-ray pathway. It is probable that the same rays are concerned in the destruction of cancer cells and that the same statement applies to the path of such x-rays through living tissues.

It is obvious that the x-rays which influence the photographic plate or the retina (fluoroscope) travel in straight lines from the target through the living tissues. These rays are limited sharply by lead or other impervious screens. The x-rays which injure superficial epithelium (hard and soft rays) are sharply limited by impervious screens.

Theoretically it is impossible to outline the track of a beam of radiation through the deeper living tissues. The following possibilities

are to be considered. (*a*) The x-rays may pass through the living tissues in a straight line with no deviation and with no diminution in strength except as they are absorbed by various cells (Duane (3)) or as they decrease in energy in proportion to the square of the distance from the target (Voltz (12)). (*b*) The x-rays may be in part dispersed by reflection and refraction on contact with the cells of the living body (Shearer (9); Rankin and Chambers (7); Wilsey (14); Rosa (8)). (*c*) The x-rays may give rise to secondary radiation which may be effective at some distance from the sources (Müller (6); Chaoul (2); Taekel and Sippel (11)) or only locally near the point of incidence (Lindemann (4); Barkla (1)). Shearer thinks that about one-fifth of the radiation is scattered sidewise in the tissues.

Lindemann (4) thinks the metallic ions, mercury, iron, calcium, etc., in the tissues give rise to secondary radiation, which affects sensitive cells. He cites the spleen, duodenum and large intestine, liver, and leucocytes. Red blood cells, bile capillaries, and liver cells, however, are not injured by quite large amounts of radiation, yet they contain or are near a large supply of metallic elements.

Müller (6) finds that the ionization power of alpha, beta, and gamma radiation decreases in the proportion of 10,000:100:1 respectively. By analogy the deep effects of gamma radiation are supposed to be enhanced one hundredfold by secondary beta-rays. Szilard (10), on the other hand, states that secondary rays have 530 times less energy than primary rays. What actually happens in cells sensitive to radiation as it passes through or is absorbed by them is a matter for conjecture as we are dealing with living organs rather than with the photographic plate.

Method.

This study is concerned chiefly with observations made upon dogs in which intestinal lesions were produced for the purpose of studying regeneration after exposure to radiation. Usually far more radiation was used than is necessary merely to destroy the epithelium. The lesions were studied at periods varying from 5 days (Dog 19–122) to 69 days (Dog 20–50) after exposure to radiation. No distinction is made between the relative states of regeneration in the "chronic lesions" but they are spoken of roughly as "denuded" or "pathological" sections or surfaces. The uninjured mucosa is designated as "normal." Only a few characteristic experiments are reported.

The same equipment was used as in the other studies (Warren and Whipple (13)). A Coolidge tube of medium focus, set at a constant

skin-target distance of 25 cm., was run at an E.M.F. of 90 to 100 kilovolts and a current strength (filament) of 8 milliamperes; 2 mm. sheets of aluminum were used throughout to filter the radiation. The amount of radiation varies somewhat in the different experiments; 2 mm. of lead-foil placed upon the skin of the abdomen was used as a screen. The apertures in the lead screen varied in size in the different experiments, some being round while others were square or rectangular.

The plane of the face of the target was at a 45° angle with the horizontal plane of the surface of the screen so that radiation advancing along a line drawn from the focal spot to the center of the aperture was always perpendicular to the plane of the screen or the skin and usually also to the longitudinal axis of the body of the dog.

The dogs were killed under ether anesthesia at different periods after radiation and the viscera examined in the gross and microscopically. An attempt was made to reconstruct the path of the radiation by putting together the injured intestinal sections in the abdomen at autopsy. The results are remarkably constant and the lesions are striking both in the gross and microscopically.

EXPERIMENTAL OBSERVATIONS.

Experiment 1.—Dog 19-127.[1] July 15, 1919. Lead-foil (2 mm.) over thorax and upper abdomen down to costal margin. Radiation 350 M.A.M. at 95 kilovolts spread diffusely over the lower abdomen.

Autopsy.—5 days later. On opening the abdomen the intestinal peritoneum was entirely negative in the upper abdomen. Below the level of the costal margin the peritoneum was injected and the intestinal walls were flabby. The distinction was sharply defined at this level. On opening the lumen of the small intestine the mucosa of the upper one-third of the duodenum and most of the jejunum was normal in appearance. The transition between the normal and the pathological sections corresponded to the transverse line at the level of the costal margin and was as sharp as if the lesion were "made by the naked cautery." It was limited microscopically to approximately two low power fields. This included the "disturbed" slightly atrophic epithelium at the edge of the lesion for a distance of about one-half a low power field.

The distinct and rather abrupt transition between the normal and injured areas may be explained by the fact that the actual area

[1] Radiation and autopsy by Dr. Irvine McQuarrie.

radiated did not cover more than the area of a 7 or 8 inch circle and there was very little chance for much blurring of the edges of the screened area. The amount of radiation expended over each area was enough to injure practically all of the intestinal epithelium directly beneath the target. This experiment illustrates the efficiency and accuracy with which a large part of the deep body tissues may be protected while another part is being exposed to intensive radiation.

Experiment 2.—Dog 20-11, small fat well developed mongrel, male; weight 16 pounds. August 22, 1919. Given 456 M.A.M. of radiation under an E.M.F. of 90 kilovolts with a current strength of 8 milliamperes over the abdomen in the upper left quadrant and midline through an aperture 6 by 8 cm. square. The lead screen was 2 mm. in thickness.

The usual symptoms of intoxication followed a few days later and then cleared up. The dog was killed with ether anesthesia on the 7th day after radiation.

Autopsy.—No abnormalities in any of the viscera except the small intestine. There were several of these lesions—one in the lower third of the duodenum, six in the jejunum, and two in the upper ileum. The edges of these pale denuded areas were clean-cut and slightly rounded and marked off from the pale velvety mucosa by a slight injection of the villi. The transition from the normal to the denuded surfaces took place in 2 to 4 mm. as in the other experiments. On longitudinal section the margins of these depressed areas were precipitous. Occasionally these areas of injury were not sharply demarcated in intestinal loops. It is probable that such areas are to be explained by a coil or loop of intestine which was situated at the margin of the cone of radiation and therefore incompletely exposed to the x-rays. Roughly over one-third of the intestine was occupied by these denuded areas. Microscopically the transition from normal to the depressed and denuded surfaces took place within the space covered by less than two low power microscopic fields.

These two experiments showed recently produced or acute lesions of the intestinal tract which were sharply limited at their edges and conformed in extent on reconstruction to the intestine which was beneath the aperture in the lead screen.

Experiment 3.—Dog 20-4. November 26, 1919. Normal, active, young mongrel, female; weight 27 pounds. The abdomen was screened by lead-foil 2 mm. in thickness. A circular aperture 7.5 cm. in diameter was cut in the lead screen so that its center was placed approximately 1 cm. below the umbilicus in the midline; 400 M.A.M. of radiation were expended over this area under an E.M.F. of 90 kilovolts. The animal developed the usual symptoms of intoxication and died 7 days later.

Autopsy.—Immediately after death. No abnormalities other than those in the small intestine. The greater part of the jejunum was in the center of the abdomen and interspersed with it were many coils of the ileum. The walls of the jejunum especially seemed thin and flabby and pouched out in little pockets. On opening up the small intestine the greater part of the duodenum except for a few small short patches in the middle section was pale, velvety, and normal. The jejunum except for a few rather short sections of pale velvety mucosa presented a dark red glassy surface suggesting absence of the mucosa. The ileum mucosa, on the other hand, was alternately pale and velvety for a few inches and then dark red and glassy for 1 to 3 inches. The edges of these denuded sections were sharply demarcated, the transition occurring in 2 to 3 mm. About one-half of the total length of the small intestine was denuded of mucosa in this experiment. Microscopically these sections showed a transition from normal to the denuded area practically within the width of one low power microscopic field.

The lesions could hardly have been more sharply differentiated by the naked cautery. The denuded areas of greater length than the diameter of the aperture in the lead screen can readily be explained as occurring in those coils of intestine lying well within the 3 inch circle and not extending straight across it as some of the others do. On reconstruction at autopsy these lesions corresponded to the cone of radiation extended down through the depths of the abdomen.

Experiment 4.—Dog 19-21, normal, active old mongrel, female; weight 34 pounds. September 4, 1919. The abdomen was screened by lead-foil 2 mm. in thickness. An aperture 5 cm. in diameter in the lead screen was fixed at the level of the umbilicus in the midline. This area was exposed to 450 M.A.M. of radiation at an E.M.F. of 90 kilovolts. The dog was sacrificed on the 12th day under ether anesthesia.

Autopsy.—The skin had not broken down in the radiated area. The viscera were entirely negative, except for short isolated coils of intestine in the middle of the abdomen roughly in or near the midline. These abnormal parts were thin walled. The peritoneum over them was slightly rough and injected. They were distributed as follows: one in the lower duodenum, three in the jejunum, and two in the ileum. All were approximately 5 cm. in length though the intestinal coils shortened up somewhat on being exposed to the air. On opening the intestinal lumen the mucosa was seen to be pale and velvety to the edge of these sections. The sections themselves were slightly depressed, with a whitish somewhat granular surface. The transition at the edge to the normal mucosa occurred in a few millimeters and was sharp and very distinct. About one-quarter of the total length of the small intestine presented this abnormal appearance.

This experiment indicates that even in subacute lesions the sharp demarcation remains a characteristic of the lesions produced by the beam of radiation penetrating the deeper structures. The lack of regeneration of the mucosa in such experiments suggests the familiar skin x-ray ulcer reaction.

Experiment 5.—Dog 20-56, setter, good condition, male; weight 35 pounds. Given 480 M.A.M. of radiation at 90 kilovolts. January 8, 1920. The filter was $1\frac{7}{8}$ mm. of sheet aluminum. The aperture in 1 mm. lead-foil was 7.5 cm. square and was located over the stomach. Under the fluoroscope the stomach filled with barium more than filled this aperture.

Autopsy.—11 weeks later. There was a sharply demarcated area, denuded of epithelium, on the anterior wall of the stomach between the greater and lesser curvatures directly beneath and corresponding to the location of the skin burn, approximately 4.5 by 7.5 cm. square. The stomach wall contracted down on exposure to the air and after fixation so that we had a rather distorted picture of the lesion (Fig. 1). There were similar denuded areas in various loops of the small intestine. Microscopically the transition from the normal to the denuded areas took place within two low power microscopic fields in both the stomach and intestinal lesions.

Experiment 6.—Dog 20-50. The 5 cm. circular aperture in the lead screen was placed over the mid-abdomen. December 12, 1919. Given 480 M.A.M. radiation by the usual method.

Autopsy.—69 days later. There were seven sharply demarcated sections in the small intestine denuded of mucosa corresponding to the size of the aperture in the lead screen—none more than 5 cm. in length. The ulcer edges were sharp and the transition from the normal to the denuded surfaces of the intestinal wall took place under the microscope within two low power fields. On reconstruction these chronic lesions corresponded to a truncated cone in the abdomen with its upper surface equal in size and shape to the aperture in the lead screen.

DISCUSSION.

The skin burn, when present, and the sharply limited and circumscribed injuries to the intestinal mucosa beneath it suggest that the type of radiation capable of injuring intestinal epithelium travels out from the target along the radii of progressively enlarging spheres just as light waves travel out from a point source. It appears probable that that part of the radiation which is absorbed by each successive layer of tissues is not dispersed in all directions to act upon the same or other tissues at a distance but affects the sensitive cells, by which

it is absorbed, or such cells as may be sensitive to its stimulus as the radiation passes through them.

There has been considerable speculation as to the factors which make one cell sensitive to these hard x-rays and a neighboring cell totally inert or resistant. Metallic ions have been suggested and this is as good an example as any. Ferments were very popular a few years ago and at that time these intangible agents were held responsible for this inexplicable cell individuality in reaction to the x-ray. All these suggestions fail when we attempt to explain the known facts. Why does the x-ray injure one type of leucocyte and not another and not at all the nucleated or non-nucleated red cells? Why does the x-ray injure germinal cells but not spinal cord and ganglia and other highly differentiated cells? These questions could be multiplied but we always come back to individual peculiarity of the cell. One cell is different or similar in function and entirely different or similar in reaction to the x-ray. Even if we cannot give the correct explanation, it is of interest to the physiologist and physician to be familiar with these individual peculiarities of body cells in their reaction to the hard x-rays. The physician should be able to avoid dangerous therapeutic exposure.

The most convincing lesion was found in the first portion of the duodenum—compare Experiment 1—and this observation has been repeated many times in different experiments. The first part of the duodenum is fixed fairly securely in the deepest part of the dog's abdomen just under the costal margin. Any beam of hard x-rays which injures its mucosa must have passed through skin, muscle, peritoneum, and several coils of small intestine. Yet we observe in the duodenum a clean-cut edge marking the normal from the destroyed mucosa. It seems to us that this indicates that these hard x-rays travel in straight lines through these various living tissues and that the secondary radiation which presumably is formed has no power to injure these mucosal cells or other body cells.

Martin and Rogers (5) have recently published pictures of lesions of the intestinal tract which were sharply defined by the use of screens, but they do not discuss the probable significance of this condition.

Intestinal motility is related to certain observations in these experiments. We are familiar with peristaltic activity in the stomach,

duodenum, and colon without any considerable change in the position of these viscera. The average physician thinks of the small intestine as combining peristaltic activity with considerable change in position of its coils or loops. Our experiments would indicate that in spite of active peristalsis there is very little if any change in position of various coils of the ileum and jejunum. How else may we explain these clear-cut ulcers in the small intestine of dogs exposed to hard x-rays over periods of 2 to 4 hours? Some of these animals were given morphia before radiation and there was clinical evidence of increased peristaltic activity as is usually caused by morphia in dogs. Many dogs were radiated without morphia and the findings were identical. It was often necessary to continue the x-ray exposure over 2 to 4 hours, including the time necessary for cooling the tube. If these loops of small intestine developed any change in position associated with peristalsis we would confidently expect much blurring of the zones of injury or even a diffuse injury of the small intestine. On the contrary we find a series of clear-cut areas of injury in the small gut corresponding to the cone of radiation as limited by the lead screen. All evidence then is against there being any change in position of the loops of jejunum and ileum in spite of active peristalsis during the period of radiation (2 to 4 hours).

SUMMARY.

X-rays which injure intestinal epithelium (and presumably other body or tumor cells) travel in straight lines from the target through the living tissues, forming a cone or beam of rays as controlled by impervious screens.

It is probable that secondary radiation is formed, especially deep in the body tissues, but such radiation does no injury to intestinal epithelium outside of the cone or path of radiation.

Lesions in the stomach and intestine may be confidently predicted from a knowledge of the size and form of the cone or beam of x-rays given over the abdomen. These lesions even more than skin burns do not heal and may in fact go on after many weeks to perforation.

Even in the depths of the abdomen the duodenal lesions are as clean-cut as a peptic ulcer, indicating the lack of dispersion or scattering of the primary or secondary rays in passage through the living

tissues. Transition from normal to necrotic mucosa rarely occupies more than 2 to 3 mm. and often can be observed in a single low power microscopic field.

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EXPLANATION OF PLATE 45.

FIG. 1. Stomach "ulcer"—77 days after receiving 480 M.A.M. of radiation—8 milliamperes at an E.M.F. of 90 kilovolts. The skin-target distance is 10 inches with a 1⅞ mm. aluminum filter. The body screen aperture is 7.5 cm. square. The ulcer is 4.5 by 7.5 cm. and is sharply punched out. The edges are rolled. The exposed muscle is brownish and granular and edematous. A well organized clot stands out from the upper corner. The skin burn is similar in size and shape. Fluoroscopy at 5 weeks demonstrated a defect on the ventral stomach wall.

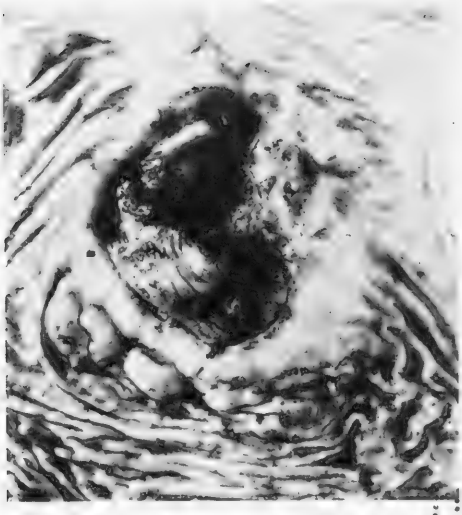

FIG. 1.

(Warren and Whipple: Roentgen ray intoxication. III.)

ROENTGEN RAY INTOXICATION.

IV. Intestinal Lesions and Acute Intoxication Produced by Radiation in a Variety of Animals.

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When our x-ray experiments on dogs gave such uniform results both as to intestinal injury and clinical intoxication we became interested in the reaction to radiation of other common laboratory animals. We found that in a great variety of animals the intestinal epithelium was sensitive to the x-ray; indeed capable of injury and destruction by means of radiation with a clinical reaction due to a disturbed intestinal tract.

The series of animals given below is not sufficient to establish the lethal dose and intestinal pathology as accurately as has been done for the dog (Warren and Whipple (13)) but there is striking similarity which strengthens our belief in the fragmentary data indicating that human beings are likewise sensitive to these hard x-rays. The roentgenologist must be on his guard to recognize the evidence of this condition in human beings and having established toxic doses he must guard against permanent injury of the intestinal mucosa. The chronicity of the intestinal ulcerations is no less disturbing than that of the familiar x-ray skin burn, or ulcers.

A review of the experimental work done upon laboratory animals with various types of radiation is very confusing. There are several reasons for this. The most obvious is that there has been the greatest variety as to the source or amount of radiation used. The second obvious factor is the individual resistance or lack of sensitivity of different animals to various amounts and kinds of radiation. Most of the earlier investigators were interested in the effects of the softer

radiations from the lower powered Roentgen ray machines upon the skin, generative organs, spleen, and bone marrow.

Frogs and young amphibia have been used frequently. Fromme (3) found that 60 to 270 M.A.M. killed a young axolotl in 4 days. No reference to the use of reptiles was found.

Birds were exposed to radiation by Krause (1906 (5)) and by Perthes (1903 (9)) who reports that one chick died in 22 days after radiation.

Lazarus-Barlow (1920 (6)) in a preliminary report described changes in the intestinal mucosa and the general organism of rats, rabbits, cats, and dogs after exposure to large amounts of radium. The changes seem comparable to our experimental results with the Roentgen rays in the same animals. Meyer and Ritter (8) were the first to establish the lethal dose for mice—30 x (hard BW5 radiation)—4 to 10 days were the various antemortem periods. Many have noticed the intoxication with diarrhea, etc., but all save a few of the more recent authors (Fromme (3); Lazarus-Barlow (6); Denis *et al.* (1)) have neglected to make histological studies of the animals to discover the cause.

Rabbits were used by Warthin (11, 12), Perthes (9), Denis, Martin, and Aldrich (1), and Hussey (4). One given 600 M.A.M. by Warthin died 40 hours later. This is somewhat earlier than was to be expected. The experiments of Denis *et al.* show that for rabbits the equivalent of 300 M.A.M. over the abdomen at 10 inches is just below the minimal lethal dose. They found changes in the alkali reserve during the intoxication following radiation. Hussey (4) thought that 150 M.A.M. at 6 inches was the maximal sublethal amount for rabbits. He noted an alkali excess which paralleled the drop in leucocytes, especially the mononuclears.

Dogs were used by Regaud, Nogier, and Lacassagne (10) in the best piece of experimental work reported in the earlier literature. They described the more chronic lesions in the intestines and stomach. The significance of their findings has not been fully appreciated. Krause (5) and Warthin (11, 12) also used dogs. Their results are hardly useful in calculating a M.L.D. as their series of exposures is not uniform nor fully described. This is a common fault observed in much of the earlier work. Emsmann (1911 (2)) reports a diarrhea in a dog 2 days after ingesting 5,000 maché units of thorium X in water, followed by death on the 4th day. No changes were noticed in the intestine. This seems within the M.L.D. when compared to our experiments. Martin and Rogers (1923 (7)) found 200 M.A.M. (about 75 kilovolts) fatal to a dog by the 5th day with the usual symptoms.

Method.

In these experiments very large amounts of radiation have been applied in single doses over the abdomen or the whole body of various laboratory animals in the endeavor to destroy a sufficient amount

of intestinal epithelium to bring about total intoxication. The smallest dose which will kill the animal in the shortest space of time has been designated as the minimal lethal dose (M.L.D.) for this type of radiation. The same standard equipment and technique were used in these experiments as in all the other studies. The target-skin distance was always 25 cm. and the filter 2 mm. of aluminum. The spark-gap was between 21 and 23.7 cm. The E.M.F. varied between 85 and 95 kilovolts. The current filament was usually 8 milliamperes and the dose was computed in milliampere minutes.

EXPERIMENTAL OBSERVATIONS.

Dogs.—In a series of sixteen dogs the average minimum lethal dose for 20 to 40 pound dogs was found to be 350 M.A.M. It is very common for dogs of this size to survive 320 M.A.M. but with evidence of severe intoxication. Much larger doses of radiation than 350 M.A.M. do not shorten the antemortem period of 4 to 5 days nor increase the severity of the intoxication syndrome—vomiting, tarry or bloody diarrhea, prostration, and death. At autopsy the mucosa of the small intestine is roughened, dark red in color, and quite thin. Histologically the villi show absence of the covering epithelium and the crypts are filled with exudate and injured and necrotic epithelium,—signs of epithelial regeneration are apparent; mitotic figures are frequent; ruptured capillaries are conspicuous. With small x-ray doses the destruction of the epithelium is apt to be patchy. With large doses it is widespread. The progress of this injury from 2 hours after radiation until the death of the animal has been reported fully in previous studies (Warren and Whipple (13)).

The erythema dose at 95 kilovolts is approximately 90 to 100 M.A.M. at 10 inches skin-target distance with a 2 mm. aluminum filter.

Cats.—Cat 19-4c, weight 4 kilos; adult male. 320 M.A.M. radiation administered over abdomen produced a moderate intoxication on the 3rd day. This increased on the 4th and 5th days and was accompanied by some diarrhea and drooling. The cat was completely recovered by the end of the week. The stools continued to be formed after this time. 3 weeks after radiation the hair fell out over a part of the right thorax and abdomen, and the skin became pigmented. The cat steadily lost weight and at the end of 7 weeks weighed only 2½ kilos. It was killed under ether anesthesia. At autopsy the viscera were essentially negative except that the mucosa of the small intestine appeared to be thinner and more granular than usual. There were no ulcerations in evidence. Microscopically, however, pronounced injury and destruction of the villi could be seen in many areas of the small intestine. There was rather diffuse injury to the crypt epithelium in the mucosa of the duodenum, jejunum, and ileum. Attempts at regeneration were atypical and these epithelial cells were pale and somewhat basophilic. In several places the muscularis mucosæ was bare. The muscle wall was atrophic

and thin, showing that this dose had caused some injury to the smooth muscle of the intestine as well as to the epithelium. The spleen and lymph follicles showed necrotic centers in the Malpighian bodies and atrophy of the pulp elements.

This is a sublethal amount of radiation producing widespread patchy injury to the small intestine which was demonstrable at the end of 7 weeks. This injury to the mucosa was probably responsible for the conspicuous loss of weight.

Cat 19-1c, weight 1¼ kilos; young female. 400 M.A.M. radiation was administered over the abdomen at 90 kilovolts with a 2 mm. aluminum filter. The animal appeared intoxicated on the 3rd day and was found dead on the 4th. No diarrhea or vomitus. Autopsy showed no gross abnormalities except in the small intestine. The mucosa, especially in the duodenum, upper jejunum, and middle ileum, was diffusely injected and dark red in color. The surface was still velvety and there were no ulcerations. There were quite a few live segmented worms present. The mucosa of the stomach and colon was pale and apparently normal. The feces in the colon were fluid though not blood-stained.

Microscopically the duodenum, jejunum, and ileum showed marked and widespread collapse of the crypts. The villi where present were short, thick, and clubbed. The epithelial cells remaining in the crypts were large, pale, and tended to take the basophilic stain. Mitoses were frequent. There was some infiltration of the submucosa with a few leucocytes and phagocytes. Ruptured capillaries contained fibrin plugs in many cases. The spleen and lymph follicles showed pulp atrophy and phagocytosis of the degenerating nuclei. The mature ova were necrotic while the immature ova were unchanged.

This is a lethal dose for a rather small cat. A smaller amount, probably 350 M.A.M., would probably have been lethal. The injury to the intestinal mucosa is marked and rather widespread.

Cat 19-3c, weight 2½ kilos; adult female. 480 M.A.M. radiation was administered over the abdomen. On the 2nd day the cat became definitely intoxicated. Vomitus and tarry stools appeared that afternoon. The cat was very sick on the 3rd day and was killed under ether. It was evident clinically that the cat would die early on the 4th day. Autopsy showed a pale, slightly granular mucosa in the small intestine with the exception of the lower ileum where there were many slightly depressed hemorrhagic areas. There was no extensive denudation of the mucosa evident in gross and the appearance did not deviate much from the normal except for the hemorrhagic areas. Microscopically, however, the crypt epithelium had suffered marked injury in all of the intestine except a portion of the ileum and the colon, where was noted only slight injury. The Malpighian bodies of the spleen and lymph nodes showed pale hypertrophied centers. There was a slight amount of pulp atrophy with much phagocytosis going on. The remainder of the viscera were negative.

This amount of radiation is excessive for a lethal dose. These lesions are exactly comparable to those in a dog sacrificed on the 3rd day following a large lethal dose of radiation.[1]

[1] Warren and Whipple, Paper I, this series.

Rabbits.—Two rabbits given 400 and 600 M.A.M. respectively died on the 7th and 13th days. The first one (No. 19-1r) was quite intoxicated on the 2nd and 3rd days and lost 2 pounds in weight in the next few days before death. At autopsy nothing abnormal could be made out in the mucosa of the intestine. Histologically not only was there patchy destruction of the mucosa of the small intestine but there was also a widespread injury to the epithelium of the cecum. The picture resembled that described in the other animals.

This dose of radiation is very near the M.L.D. The animal was very sick on the 2nd and 3rd days and barely pulled through each day up to its death 4 days later.

The second rabbit (No. 19-2r) though given the larger amount of radiation (600 M.A.M.) did not show the intoxication, loss of weight, and marked intestinal injury that the first one did. The result was probably due to a decreased amount of radiation as the machine was found several days later to be defective. The lesions, however, were similar to those in the other rabbit though less marked in extent.

Guinea Pigs.—Guinea Pig 19-2g, weight 525 gm.; adult female. Received 228 M.A.M. radiation over the whole body apparently without any immediate effect. 6 days later the guinea pig had a greenish diarrhea which later became black. During the last 10 days the guinea pig lost weight rapidly and became inactive. It was sacrificed on the 25th day following radiation. The hair began to fall out on the 11th day after radiation. At autopsy nothing abnormal could be made out except for fatty infiltration of the liver. The intestines were rather thin walled and flabby but gave no evidence of ulceration. Microscopically the crypts of the cecum and to some extent those of the duodenum and colon showed large pale atypical cells. There was no destruction of the epithelium of the villi.

It is interesting that these large, pale, distinctly abnormal crypt cells seen in the duodenum and colon resembled similar cells seen in dogs 2 days after radiation. This amount of radiation is just short of the M.L.D.

Guinea Pig 19-3g, weight 500 gm.; white, adult female. 400 M.A.M. radiation given over the whole body. The guinea pig remained apparently normal until the 4th day when it became toxic and developed a tremor of the limbs and head. It seemed very sick. There was no diarrhea. The guinea pig was found dead the following morning. At autopsy the mucosa of the small intestine was brick-red in color and covered here and there by a thick tenacious mucus. The villi seemed absent, leaving a rather raw surface which no longer presented a velvety texture. The cecum and colon were not abnormal; their mucosa was greenish and velvety. The remainder of the viscera were normal. Microscopically the epithelium of the small intestine was almost all destroyed, leaving a few epithelial cells here and there in the collapsed crypts. The epithelial cells of the colon mucosa appeared larger and paler than normal. There was no destruction of the colon villi. The other viscera were normal. This somewhat excessive dose produced a marked and widespread destruction of the epithelium of the small intestine.

Rats.—Six rats (Nos. 1 to 6) were given 100 M.A.M. radiation and one was sacrificed every day. There were no signs of intoxication and no gross lesions. Microscopically, however, the crypt cells of the duodenum, jejunum, and cecum were large and pale with large round pale nuclei containing one to two nucleoli. They were most evident and prominent in the small intestine of the rat killed on the 5th day. There was no distortion or change in the shape of the villi. The spleen contained somewhat more pigment than usual in the 5th and 6th day rats.

Six rats (Nos. 7 to 12) were given 200 M.A.M. of radiation and one was sacrificed every day. They developed a diarrhea and looked sick from the 3rd day on, and the last rat was found dead on the morning of the 6th day. Nothing could be made out in gross except in the 5th and 6th day rats whose small intestines in places presented a red or hemorrhagic mucosa. Microscopically there was evidence of abnormality in the crypt cells of the duodenum on the 1st day after radiation. The crypt cells were large and pale and increased in number. On the 2nd day there was evidence of degeneration and regeneration so that though the crypts were somewhat collapsed they were lined by sheets of large pale crypt cells. From the 2nd day on the injury to the villi was more and more evident until it reached the climax on the 6th day when the duodenal villi were stubby and the crypts markedly distorted and lined with atypical cells. The ileum showed ruptured blood vessels in great numbers. The spleen became more and more pigmented up to the 6th day. The ova were necrotic after the 3rd day and the spermatozoa after the 5th day.

A third set of six rats (Nos. 13 to 18) was exposed to 300 M.A.M. and one was sacrificed every 24 hours for 6 days. The symptoms of intoxication with diarrhea appeared on the 4th day and the sixth rat was found dead on the morning of the 6th day. Autopsy revealed several reddish patches in the small intestine in the 4th, 5th, and 6th day rats. Microscopically marked changes were noted on the 1st day following radiation in the crypt cells of the duodenum. These continued to increase in extent from this time on. The injury was very irregular in distribution and was not constant throughout the intestines, nor in the sections, from animal to animal. The fifth rat, for instance, showed remarkable destruction of the crypt epithelium with collapse of the villi in the small intestine. The cells were large and pale and spread out in thin layers over the abnormal villi. The 6th rat, which had died, showed no injury in the sections taken, though in gross there were the same red raw looking sections especially in the ileum. This patchy injury has been noted in other animals in lethal experiments. This is a large M.L.D. while 200 M.A.M is evidently a small M.L.D., the average being somewhere between.

A fourth series of five rats (Nos. 19 to 23) was exposed to 400± M.A.M. radiation and one sacrificed every day. Diarrhea and intoxication appeared on the 3rd day and the 5th day rat was not sacrificed and found dead on the 8th day. Nothing was noted in gross. Microscopically all the rats which had had diarrhea (Nos. 21, 22, and 23) showed marked injury to the duodenum with destruc-

tion and collapse of the villi. The epithelium remaining was atypical and made up of sheets of large pale cells with large circular nuclei containing usually two nucleoli. The injury was very irregular. In some sections the crypt cells were very heavily stained and murky, though intact, while the transition cells between the tips and crypts were growing rapidly and often piled up in two or three layers. These cells were large and pale and with large pale round nuclei showing one to two nucleoli.

It was found that there was a short circuit in several of the secondary coils of the transformer so that we are certain of neither the amount nor the quality of the radiation these rats received (Rats 19 to 23).

One rat (No. 24) given 600 ± M.A.M. developed a profuse brown watery diarrhea on the 3rd day and died early in the 4th day. Autopsy showed the mucosa of the ileum to be red and engorged for about one-half its length. Microscopically the duodenum and ileum showed profound destruction of the villi with only a few epithelial cells left here and there in the crypt remnants. There were several ruptured capillaries all nicely thrombosed seen in sections. The pulp of the spleen and the lymph nodes was almost completely absent and the sinuses were filled with blood. Even with this large dose the intestinal injury was rather patchy and irregularly distributed. This is probably the shortest antemortem period for rats. Usually for doses nearer the M.L.D. the antemortem interval seems to be 5 to 6 days.

In brief, the rats show the same clinical syndrome—intoxication, diarrhea, and death—and the same histological picture of destruction of intestinal epithelium, that dogs do. The crypt cells too are most severely injured. None of these animals showed evidence of septicemia with the development of purulent or necrotic foci in various organs.

Birds.—Pigeon M-2b received 300 M.A.M. radiation on March 17, and again on April 14, 1919. A watery diarrhea developed about 5 days after the first exposure and continued throughout the rest of the experiment. On May 6 the pigeon was given 400 M.A.M. and on May 20, 600 M.A.M. more. The bird died 4 weeks later. This bird received within a period of 9 weeks 1,600 M.A.M., the last two allotments of which were directed at the abdomen primarily. There was a slight amount of anemia following each exposure due probably to the few cubic centimeters of blood taken from the wing vein daily for blood counts. There was no change noted at any time in the nucleated red corpuscles. The white count dropped somewhat after each exposure and then returned almost to the previous figures. Clinically the bird was more affected by the first exposure of only 300 M.A.M. than by any of the others. Even then it was only slightly less active than usual. The diarrhea was at no time blood-stained but was mostly made up of watery or mucoid material. At autopsy nothing abnormal could be made out in gross. Histologically, the villi of the duodenum were long and filamentous. The epithelium seemed intact and unchanged. A few of the crypts in the gizzard at its lower end were lined by atypical pale thin cells. There is a surprising lack of evidence of injury done this pigeon by radiation.

No. 19-3b, a large hen, was given 350 M.A.M. over the abdomen and was found dead 16 hours later. At autopsy it was thought that the mucosa of the duodenum was somewhat injected. Nothing else abnormal was seen. Histologically the nuclei of the crypts in the mucosa of the small intestine were all large, pale, and with their chromatin in masses they appeared pycnotic. Around the crypts in the interstitial tissue were masses of chromatin evidently from disintegrated cells. The cells lining the crypts were still in place. There was no polynuclear cell reaction. This hen was active, fat, and apparently in good condition when she was given radiation. The early fatality we are unable to explain. The changes in the intestine of this fowl and the next bird are similar to early changes reported in dogs.[2]

A pigeon (No. 19-5b) was given 800 M.A.M. radiation over the abdomen. The feces became fluid the next day and continued so. On the 6th day the diarrhea became profuse and on the 11th day there was a rapid loss of weight and profuse watery diarrhea. The bird looked very sick and sat with its feathers ruffled up. At autopsy there were no evident abnormalities other than marked emaciation. Histologically only one section of the duodenum showed destruction of the villi with atypical cells in the injured crypts. Another section showed a rather atypical pale and irregular epithelium covering stubby rounded villi. This bird though given much more than the others seems to have received a sublethal amount. Perhaps 100 or so M.A.M. more would approach the M.L.D. more closely. Study of the red corpuscles in this bird demonstrated no changes.

Reptiles.—A group of five grass snakes was exposed to various amounts of radiation up to 900 M.A.M. without any effect except that they became rapidly emaciated. One, receiving 800 M.A.M. a week after the changing of its skin, died in 20 days. Nothing abnormal could be detected in the mucosa cells of this snake nor in those two given 900 M.A.M. and sacrificed 26 days afterward.

Amphibians.—A series of five adult frogs was given 560 M.A.M. of radiation. One sacrificed on the 2nd day showed slight changes in the cells of the crypts of the duodenum and colon. Another sacrificed upon the 3rd day showed the same cells to be pycnotic and to have multiplied somewhat. The cells were large and pale and there were generally one or two large nucleoli in the large pale nuclei. These cells stand out prominently from the normal cells which are smaller, more cuboidal, and contain very densely staining oval nuclei. The remainder of the frogs were kept 3 weeks longer without showing any symptoms. One of these was then exposed to 800 M.A.M. 24 days after the previous radiation. It died 19 days later after showing tremors, ataxia, paralysis, and a wrinkled, dried up skin. Autopsy done several hours after death showed a remarkably advanced autolysis which obscured the microscopic picture completely. The other two frogs continued active and well and showed no abnormalities on sacrifice 1 month later.

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[2] Warren and Whipple, Paper II, this series.

TABLE I.

Roentgen Radiation.
*The Minimum Lethal Dose.**

Animal.	No. of animals.	M.L.D.	Onset of symptoms of intoxication.	Death after radiation
		M.A.M.	*days*	*days*
Frogs.†	3	Over 800	3–5	18–20
Snakes.†	5	" 800	4–8	18–20
Birds.†	4	" 800	4–5	10–11
Rats.	24	200–300	3–4	5–6
Guinea pigs.	3	300–350	3–4	4–5
Rabbits.	2	350–450	2–3	4–5
Cats.	3	350–400	2–3	4
Dogs.	16	350	2–3	4

* 95 kilovolts; 8 milliamperes; 10 inch spark-gap; 10 inch skin-target distance; 2 mm. aluminum filter. The amounts of radiation and the intervals of time indicated represent the average.

† 800 milliampere minutes was the most radiation given any single animal at one time. It is evidently somewhat less than the M.L.D. for frogs, snakes, and birds.

TABLE II.

Comparative Dosage for Sensitive Tissues.*

Dose.	Experimental values.			Calculated average values.			Erythema dose for man according to Seitz and Wintz.†
	Guinea pig.	Cat.	Dog.	Guinea pig.	Cat.	Dog.	
	M.A.M.	M.A.M.	M.A.M.	*per cent*	*per cent*	*per cent*	*per cent*
Erythema dose.‡	175	300	100	100	100	100	100
Light intestinal injury.	220	320	100	120	107	100	135
Muscle dose.§	—	320	200	—	107	200	180
M.L.D.	300–350	350–400	350	200	117	350	?

* 90 to 100 kilovolts; 8 milliamperes; 10 inch spark-gap; 10 inch skin-target distance; 2 mm. aluminum filter.

† Seitz, L., and Wintz, H., *Münch. med. Woch.*, 1918, lxv, 89.

‡ The erythema dose in laboratory animals is the amount which causes superficial desquamation of the skin; this is less than that producing a first degree burn and greater than that causing depilation.

§ The muscle dose is the smallest amount which produces atrophy of the fibers of the smooth muscle of the intestinal wall.

DISCUSSION.

The experiments given above show that the common laboratory mammals are sensitive to the x-rays in much the same dosage, and with similar clinical reaction and relatively uniform intestinal pathology (Tables I and II). Guinea pigs and rats are a little more susceptible to the radiation than are dogs, cats, and rabbits. The M.L.D. is correspondingly less in rats and guinea pigs. We have no evidence that the intoxication and intestinal injury are modified by body weight or body surface. We note little if any difference between a dog of 20 or 40 pounds in weight and a cat or rabbit of 4 to 8 pounds.

By contrast with the above group of mammals we observe that birds, frogs, and reptiles are very resistant to radiation. These animals can tolerate with little disturbance 1 or even 2 M.L.D. of radiation for the dog or cat. This peculiar resistance of birds, frogs, and reptiles is worthy of further study. We regret that our experiments are not adequate to give the answer to this riddle. One's first thought is that the feathers of birds and the skin of snakes may be very resistant to the x-rays and act as efficient screens. There is some experimental evidence to support this suggestion but no evidence that the frog's skin is impenetrable to the x-rays. The histology of these exposed animals indicates a less acute and extensive injury of intestinal epithelium by the longest x-ray exposures (800 milliampere minutes). It seems probable that the intestinal tract of these animals differs fundamentally in its sensitivity to x-rays as compared to the dog or cat.

We hoped that these enormous doses of x-rays might bring out interesting changes in the nucleated red corpuscles of the birds but can only report negative observations. Our experiments indicate that the white blood cells of birds react to the x-ray exposures in somewhat the same fashion as do the white blood cells of the dog and cat. The red blood cells of birds in spite of the rich chromatin deposit in the nucleus are not injured by the x-ray and escape as completely as do the non-nucleated red cells of the dog and cat.

The whole question of repair of the intestinal epithelium following an x-ray injury is deserving of much study and is fascinating to the histologist. At times the repair seems to be complete with restora-

tion to normal but with longer doses healing may be long delayed and permanent ulcers may be formed which show no evidence of healing for months and may in fact lead to intestinal perforations. The x-ray offers a simple means of producing ulcers of the stomach, intestine, or colon which will resist healing as do peptic, duodenal, or other chronic ulcers of the intestinal tract.

In these experiments we note differences in sensitivity to the x-rays in different parts of the intestinal tract. For example, the cecal mucosa of the dog is relatively more resistant than is that of the small intestine. In the rabbit the cecal mucosa is quite extensively injured and seems as sensitive to radiation as the small intestine. We may recall that the physiology of the cecum in these two animals is vastly different and this may explain the differences in sensitivity to the x-ray.

One is impressed by the patchy injury of the intestinal tract noted in dogs dying from a M.L.D. of radiation. Larger doses give a much more uniform distribution of the intestinal injury. We may choose to explain this on the basis of the intestinal contents or the position of various intestinal coils in relation to the cone of radiation or on the score of intestinal activity, etc., but the fact remains that there is no convincing explanation for this observation at present.

SUMMARY.

These experiments show that the common laboratory animals are about equally sensitive to the x-ray given over the abdomen. The clinical reaction following a M.L.D. is very similar and the intestinal pathology almost identical. The rat and guinea pig are slightly more sensitive to the x-ray than are the dog, cat, and rabbit.

By contrast birds, frogs, and reptiles are very resistant to the x-ray and may tolerate two or three doses of radiation lethal for dogs. We can offer no convincing explanation for this fact which is discussed above.

These data strengthen our belief in the scattered and incomplete observations on human cases which indicate that the human intestinal tract is likewise sensitive to radiation. This fact must be given careful consideration in conditions where abdominal or pelvic radiation is being used because such injury done to intestinal epithelium is always serious and in some cases irreparable.

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